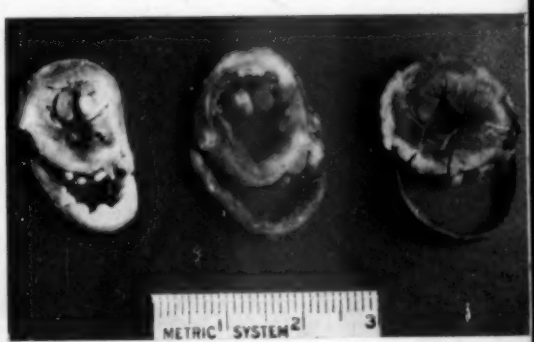
A.M.A.

Archives of PATHOLOGY

JULY 1956

VOLUME 62 NUMBER 1

From Thomas et al., p. 55



AMERICAN MEDICAL ASSOCIATION Publication

TABLE OF CONTENTS

VOLUME 62 JULY 1956 NUMBER 1

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A. ARCHIVES OF PATHOLOGY

VOLUME 62 JULY 1956 NUMBER 1

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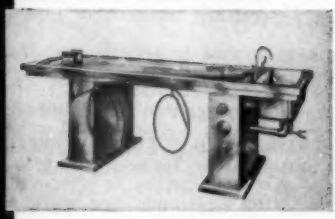

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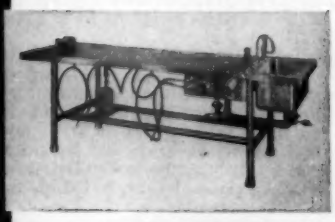

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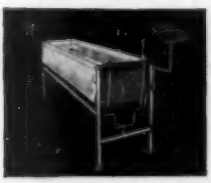

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Hepatic Duct Carcinoma Seventeen Years After Injection of Thorium Dioxide

JAMES C. ROBERTS JR., M.D.
and
KENNETH E. CARLSON, M.D., Pittsburgh

Thorotrast * is a stabilized emulsion of thorium dioxide. Although it is an excellent contrast medium for radiography,† two important factors contraindicate its use. It is the progenitor of a series of radioactive elements,[4] and it is retained indefinitely in the reticuloendothelial system.‡ Recent reports indicating that the combination of radioactivity and indefinite retention may lead to necrosis and fibrosis at injection sites, hemopoietic derangements, and neoplasms have been reviewed by Looney.§

We are reporting on a patient with carcinoma of the hepatic duct which developed 17 years after thorium dioxide was used for vasculography. In this case the presence of thorium dioxide was diagnosed during life and was proved at autopsy. Spectrochemistry was used to prove the presence of thorium dioxide, and quantitative radiation detection was used to measure the amount of residual thorium dioxide.

Submitted for publication March 1, 1956.

From the Department of Pathology of the University of Pittsburgh School of Medicine and the Presbyterian Hospital, Woman's Hospital, and Eye, and Ear Hospital.

* Supplied by Testagar & Company, Inc., Detroit, as 24% to 26% stabilized colloidal thorium dioxide by volume, 25% aqueous dextrin, with 0.15% methylparaben (Methyl Parasept) as preservative.

† References 8 and 11.

‡ References 6, 8, and 11.

§ References 5, 6, and 7.

Report of a Case

Clinical History.—The patient was a 45-year-old white woman at the time of her death, July 7, 1955. At the age of 4 years she fell and injured her right lower leg. Over the subsequent years she developed localized edema and prominent varices at the injury site. At the age of 28 she was admitted to a hospital for treatment of this condition. Arteriovenous fistulae were demonstrated by intravascular injection of thorium dioxide in the right lower leg. These fistulae were ligated and obliterated. After the operation she improved and was discharged. Six years later severe hemorrhage from the operative site occurred, and she was admitted to another hospital. A midthigh amputation was performed, and the postoperative course was uneventful. No thorium dioxide was observed microscopically in the amputated limb.

Ten years after the amputation and seventeen years after the vasculographic study with the thorium, jaundice, anorexia, pruritus, and acholic stools were noted. These symptoms progressed over a period of four months, and the patient was admitted to the Presbyterian Hospital in Pittsburgh, on June 13, 1955. Pertinent physical findings were intense jaundice, mild fever, hepatosplenomegaly, and ascites. All laboratory studies indicated obstructive jaundice. The ascitic fluid contained malignant cells. The diagnosis of thorium dioxide deposition was made on the basis of an upper abdominal roentgenogram (Fig. 1). A lap-

arotomy showed extrinsic obstruction of the main hepatic duct by neoplasm. She died on the 12th postoperative day.

Autopsy Findings.—Three thousand milliliters of cloudy yellow fluid filled the peritoneal cavity. The liver weighed 1150 gm. The surface was green and granular; the hilus was replaced by a 7 cm. tumor mass; the portal areas were enlarged and firm; the intrahepatic bile ducts were dilated, and several were filled with green mucoid bile. The lymph nodes of the porta hepatis were enlarged and firm and filled with yellow-gray granular material. The walls of the hepatic duct, common duct, and gall bladder were infiltrated and thickened by gray tumor. The lumen of the hepatic duct was obliterated about 4 cm. proximal to the common duct, and the remaining epithelium was rough and granular. Secondary tumor was observed in the lungs, bone marrow, spleen, lymph nodes, and adrenals. The spleen weighed 50 gm. The splenic capsule was thickened and furrowed, and the cut surface had a thin yellow reticular pattern interlacing among flecks of yellow-gray crystals.

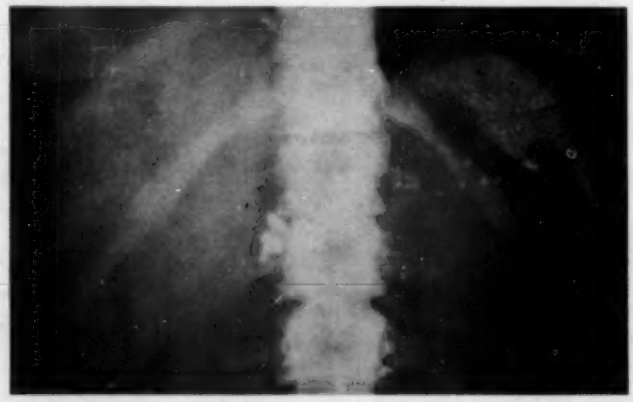

Fig. 1.—Upper abdominal roentgenogram, 18 days before death. (Presence of thorium dioxide diagnosed by Dr. L. S. Sherman, radiologist.)

Microscopic examination showed the hepatic duct to be almost completely replaced by a moderately differentiated adenocarcinoma with a scirrhous stroma (Figs. 2 and 3). The metastases were of similar histologic pattern.

The lymph nodes of the porta hepatis were filled with purple-brown granular material lying free in the tissue and in macrophages. The granules were refractile but not doubly refractile, varied in size from 2μ to 50μ, and did not appear crystalline (Fig. 4). Similar large deposits of this material were seen in the spleen (Fig. 5) and portal areas of the liver. A few granules of this material were observed in the bone marrow; none were observed in the remainder of the organs.

Special Postmortem Studies

Materials and Methods.—Spectrographic studies were performed by Dr. Mary Warga and Mr. Robert Stull, in the Department of Biophysics of the University of Pittsburgh. The samples studied included a pericholedochal lymph node from the autopsy, commercial thorium dioxide, thorium chloride, and a normal lymph node (fixed in Jöres fixative, as was the patient's node). All of these materials were powdered and burned in carbon electrodes.

The amount of residual thorium dioxide was determined as follows: Representative wet pieces of lymph node, spleen, and liver were weighed and minced with 20 ml. of distilled water. Aliquots were then counted in a calibrated scintillation crystal shielded to eliminate all but gamma rays. Commercial thorium dioxide was also counted in the same crystal, with similar geometry. Calculations for these determinations, results of which are listed in the Table, were made as follows: The figures for "counts per minute per gram" of tissue, the first column, were obtained by subtracting the background from the total counts obtained with each aliquot and multiplying the remainder by the dilution factors for each

sample to correct to 1 gm. The figures for "counts per organ," the third column, were obtained by multiplying the figures in the first column by the weights of the organs. The figures for the radioactivity of the thorium dioxide were obtained directly. The figures for the last column "counts per organ as thorium dioxide in milliliters," were obtained by dividing the "counts per organ" by the "thorium dioxide counts per milliliter."

Fig. 2.—Hepatic duct with carcinoma in wall; × 40.

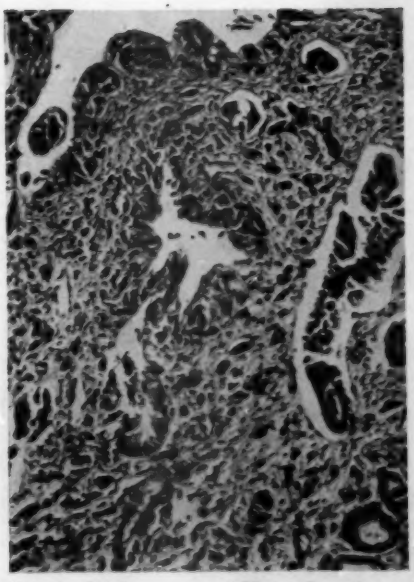

Fig. 3.—Higher power of Figure 2 to show epithelial character of neoplasm; × 150.

Autoradiographs of tissue sections of all organs were made according to Gibbs' technique.‖ Paraffin sections cut at 5μ were placed on Kodak NTB-2 plates. The plates were developed after seven days' exposure and then stained with hematoxylin and eosin. The tissue sections were not removed from the autoradiograph plates.

Results.—The spectrograph of the pericholedochal lymph node contains specific thorium lines (Fig. 6). In a few areas there are lines in the patient's lymph node that are not present in the thorium dioxide controls; however, these are present in the normal lymph node and are probably caused

‖ Gibbs, W. D.: Personal communication to the authors.

Radioactivity of Various Organs

	Counts/ Min./Gm.	Organ Wt., in Gm.	Counts/Organ	Thorium Dioxide Counts/Ml.	Counts/Organ as Thorium Dioxide, in Ml.
Liver	108	1,150	216,200	14,117	15.3
Spleen	649	50	32,450	14,117	2.3
Lymph nodes	5,333	2	10,666	14,117	0.8
Total					18.4 ml.

by some of the metals in the Jöres fixative.

It is likely that the liver, spleen, and upper abdominal lymph nodes contained most of this patient's residual thorium dioxide. Although the amount of thorium dioxide injected into this patient was not recorded, our estimated equivalent figure of 18.4 ml. of thorium dioxide retention is in good agreement with the usual dosage for vasculography of 10 to 30 ml. reported in the literature.¶

Autoradiographs showed abundant alpha

¶ References 8 and 11.

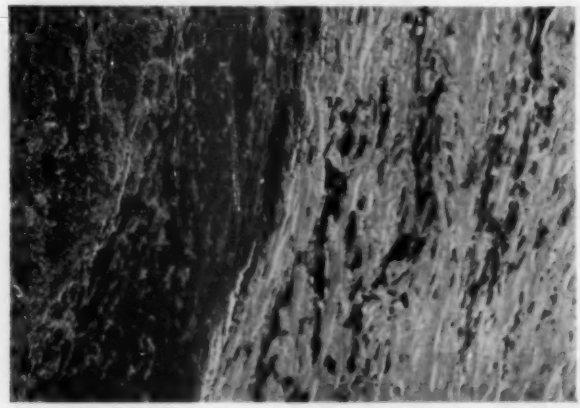

Fig. 4.—Lymph node. Architecture obliterated by thorium dioxide; × 80.

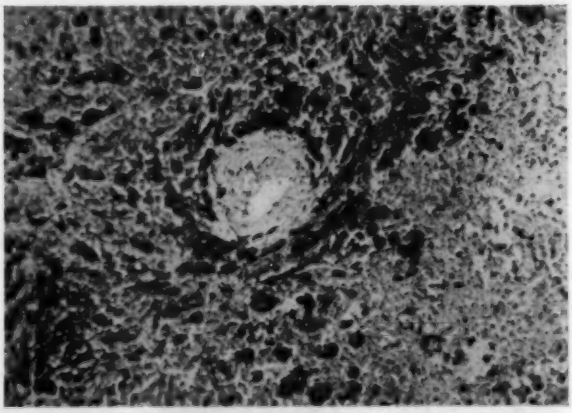

Fig. 5.—Spleen. Normal architecture obliterated. Thorium dioxide prominent. Alpha tracks localized over thorium dioxide not seen well at this power. Autoradiograph; × 80.

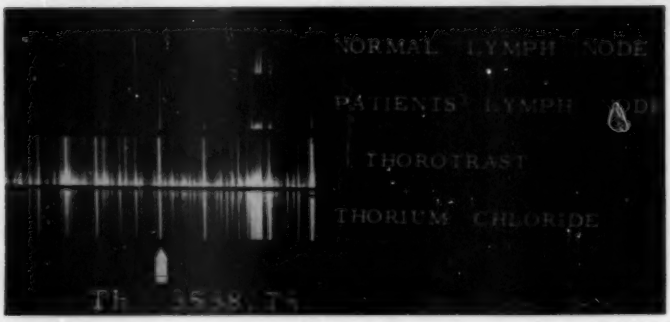

Fig. 6.—Spectrogram. Area selected for standard thorium reference line; × 4.

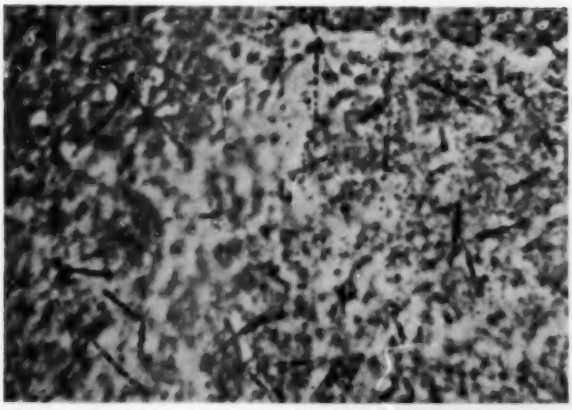

Fig. 7.—Spleen. Alpha tracks and clusters over thorium dioxide granules. Autoradiograph; × 400.

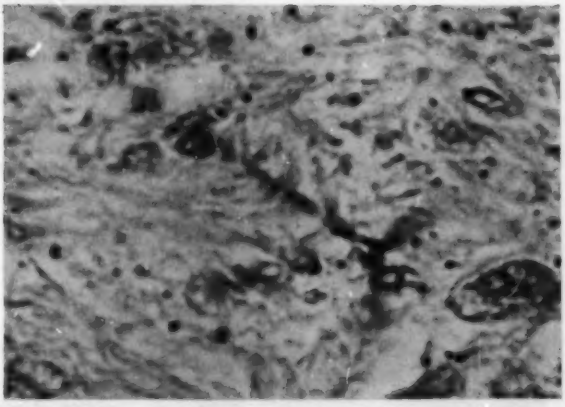

Fig. 8.—Liver. Macrophage in carcinoma stroma. Base of alpha cluster visible. Autoradiograph; × 1000.

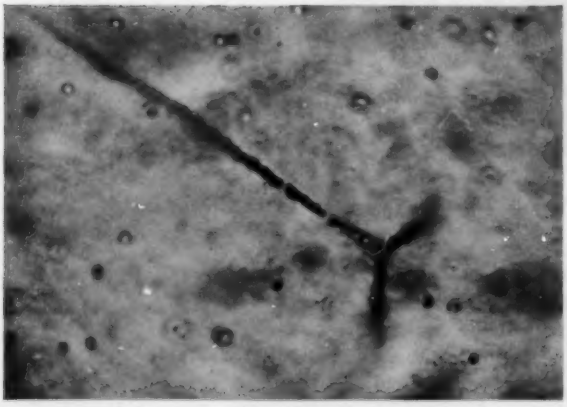

Fig. 9.—Liver. Same field as Figure 8. Focal point in emulsion. Autoradiograph; × 1000.

tracks traceable to the granular material noted in the lymph nodes, spleen, and liver (Figs. 7, 8, and 9). No alpha tracks were observed in the lungs, heart, kidney, or adrenals.

Comment

The cause-and-effect relationship of the thorium dioxide to the neoplasm of the hepatic duct, while suggestive, cannot be determined with certainty. Experimental

neoplasms produced by thorium dioxide are well documented,# and sarcomas and carcinomas in humans developing after thorium dioxide administration are being reported with increasing frequency.* However, carcinoma of the hepatic duct without history of injection of radioactive material, while rare, has been reported in 40- to 50-year-old women.† In this patient the arrangement, concentration, and radioactivity of the stored thorium dioxide were ideal for the localizing of radiation in the region of the hepatic duct (Fig. 10).

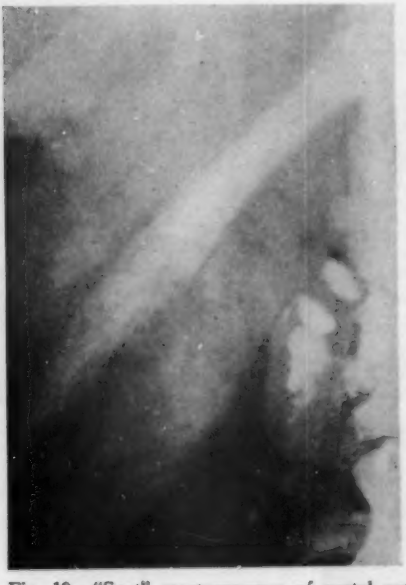

Fig. 10.—"Spot" roentgenogram of portal area. Radiopaque lymph nodes are prominent.

The concentration of the thorium dioxide in the liver and spleen is explained by the primacy of the liver and spleen as blood-clearing organs.‡ The concentration of the thorium dioxide in the upper abdominal lymph nodes is due to drainage of the liver and spleen through these nodes.

References 8, 9, and 11.

* References 2, 5-7, 10, and 11.

† References 1 and 3.

‡ References 11 and 12.

Summary

A case of carcinoma of the hepatic duct is reported. This tumor developed 17 years after intravascular injection of thorium dioxide into an extremity for demonstration of a peripheral vascular abnormality. Although no direct cause-and-effect relationship can be proved, thorium dioxide was extremely prominent in portal lymph nodes and in the portal aspect of the liver. The presence of thorium dioxide was diagnosed before death by an abdominal roentgenogram and proved after death by spectrochemistry. Radioactivity was demonstrated by gamma-counting and autoradiographs. The estimate of the quantity of thorium dioxide retained, based on calculations of the amount found at autopsy in liver, lymph nodes, and spleen, is in remarkable agreement with the usual dosage for peripheral vasculography.

Dr. J. R. Watson gave us permission to publish this case. The photography was performed by Mr. Albert Levin, F. B. P. A.

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Reaction of Normal Human Skin to Intradermally Injected Hydrocortisone

A Histologic and Histochemical Study

WILLIAM B. ATKINSON, Ph.D.
RAYMOND R. SUSKIND, M.D.
and
LEON GOLDMAN, M.D., Cincinnati

During the past decade considerable attention has been devoted to the therapeutic potentialities of adrenal cortical hormones in a wide variety of diseases not directly related to dysfunction of the adrenal cortex. Among the conditions affecting the skin, lupus erythematosus, dermatomyositis, scleroderma, pemphigus, and many other disorders have responded more or less favorably to the administration of these hormones. It soon became evident, however, that owing to the potentially dangerous side-effects which may be produced by these agents, prolonged systemic administration should be avoided in relatively benign dermatologic diseases which do not seriously affect the general health and well-being of the patient. Therefore, interest has centered more recently on the effectiveness of cortical steroids when applied topically in ointments and liquid suspensions and when injected intradermally (Goldman, Thompson, and Trice,[10] 1952; Goldman, O'Hara, and Baskett,[9] 1953). As a result of these studies, it has been found that, whereas cortisone and hydrocortisone produce similar effects when administered systemically, only hydrocortisone is effective in the alleviation of dermatologic symptoms when administered locally.

Received for publication April 20, 1956.

Department of Anatomy and Department of Dermatology and Syphilology, University of Cincinnati College of Medicine.

This investigation was supported by a grant, (A-260 2C) from the U. S. Public Health Service.

It has been well established that one of the important general actions of the cortical hormones is that of suppression of the inflammatory reaction (Dougherty and Schneebeli,[6] 1950; Dougherty,[5] 1952). In the case of dermatologic lesions, microscopic studies have revealed that within 24 hours after the intradermal injection of hydrocortisone a mass of hematoxylinophilic granular material forms in the dermis among the injected hormone crystals (Goldman, O'Hara, and Baskett[9]). Concurrently, both clinical and histologic evidence of local regression of inflammation occurs. The hormone crystals remain in situ for periods up to seven to eight months. During this time the hematoxylinophilic mass persists and clinical improvement of the lesion is maintained.

Whereas the effects of adrenal cortical hormones on the inflammatory reaction have been studied intensively, relatively little attention has been given to the local effects of these hormones on normal tissues. However, several interesting and pertinent facts have been reported. When cortisone is injected into the subcutaneous connective tissue of the mouse, morphologic evidence of damage to the fibroblasts in the area of the hormone crystals occurs within several hours (Schneebeli, Dougherty, and Loewe,[15] 1951). Topical application of either cortisone or hydrocortisone to the skin of the rat for prolonged periods results in thinning of the dermis and epidermis, inhibition of hair growth, and atrophy of the sebaceous glands (Castor and Baker,[4] 1950). Under similar conditions of hormone treatment there are also an increase in the rate and alteration in the pattern

of the dermal spreading of hyaluronidase (Hayes and Bridgeman,[11] 1951). These latter observations have been interpreted as indicating a change in the physical and/or chemical structure of the ground substance of the connective tissue. It has also been observed that the foreign body reaction is minimal around pellets of cortisone implanted in the orbital connective tissue of the rat as compared with pellets of cholesterol and sex hormones (Baker,[2] 1954). When cortisone or hydrocortisone is injected into normal human dermis, hematoxylinophilic masses develop which are similar to those occurring after the injection of these hormones into inflamed skin (Goldman, O'Hara, and Baskett[9]; Goldman,[8] 1955).

The hematoxylinophilic masses referred to above apparently represent a unique reaction of human skin to intradermally injected adrenal cortical hormones. Since their origin and composition are not known, the present histologic and histochemical study was undertaken. It was hoped that such information might also shed further light on the biologic mechanisms by which these hormones produce their local physiologic and therapeutic effects.

Methods and Material

Fourteen normal human subjects were given one or more intradermal injections of a suspension of hydrocortisone acetate or of the suspending medium alone.* The injections were made in the skin of the arm or shoulder using a standard syringe and 24-gauge needle, and their locations were marked by making India ink tattoos nearby. A total of 12 sites were injected with the hormone suspension, and individual sites were subsequently removed at intervals of ½, 24, 48, or 72 hours with a rotary punch 4 mm. in diameter. Eight sites were injected with the vehicle alone and were removed at intervals of 24, 48, or 72 hours.

The hormone preparation consisted of hydrocortisone acetate crystals, approximately 1.25μ in diameter, suspended in a sterile isotonic saline solution containing 1.5% benzyl alcohol, 0.5% sodium carboxymethylcellulose, and 0.4% polyoxyethylene. The concentration of the hormone was 25 mg. per milliliter; the volume injected was either 0.1 or 0.2 ml.

* Supplied through the courtesy of Merck & Co., Inc., Rahway, N. J.

The biopsy specimens were fixed for 24 to 48 hours in 10% aqueous formalin neutralized with 1% calcium acetate. The fixed tissues were then washed thoroughly in running water, and either frozen sections (10μ to 15μ in thickness) or paraffin sections (6μ in thickness) were prepared in the routine manner.

The tissue sections were processed by a variety of histologic and histochemical techniques for microscopic study:

Frozen Sections at All Internals After Injection.

1. Unstained sections were examined with the polarizing microscope in order to visualize the birefringent hydrocortisone acetate crystals. Parallel sections studied after extraction with lipid solvents served as controls.

Paraffin Sections at All Intervals After Injection.

1. Ehrlich's iron hematoxylin and eosin were employed for general histologic staining.

2. Basophilia, due principally to nucleic acids and acid mucopolysaccharides, was determined by staining with 0.001 M toluidine blue, buffered at pH 4.5, for one hour at room temperature. These substances were removed from control sections by extraction with 1N HCl for two hours at 37 C prior to staining (Atkinson,[1] 1952).

3. Deoxyribonucleic acid was demonstrated by the Feuglen reaction (Feulgen and Rossenbeck,[7] 1924).

4. Polysaccharides and glycoproteins were visualized by the periodic acid-leucofuchsin method. Control sections were first incubated in a solution of malt diastase to remove any glycogen that might be present (McManus,[13] 1948).

5. Free protein α-amino-acid groups were demonstrated by the ninhydrin-leucofuchsin method (Yasuma and Ichikawa,[16] 1953).

6. The possibility of free aldehyde groups being present in the tissues was investigated by treating the sections with leucofuchsin alone. These sections also served as controls for the aldehyde producers listed above (3, 4 and 5).

Frozen Sections at 24 to 72 Hours After Injection.

1. Sudan IV and Sudan B were employed as stration of the Δ-hydroxysterols, chiefly cholesterol general lipid colorants.

2. Phospholipids were visualized by Baker's acid hematin method (Baker,[3] 1946).

3. The digitonin reaction of Windaus (Lison,[12] 1936) and the Lieberman-Burchard reaction (Romieu,[14] 1925) were employed for the demonstration of the Δ-hydroxysterols, chiefly cholesterol and its esters.

Observations

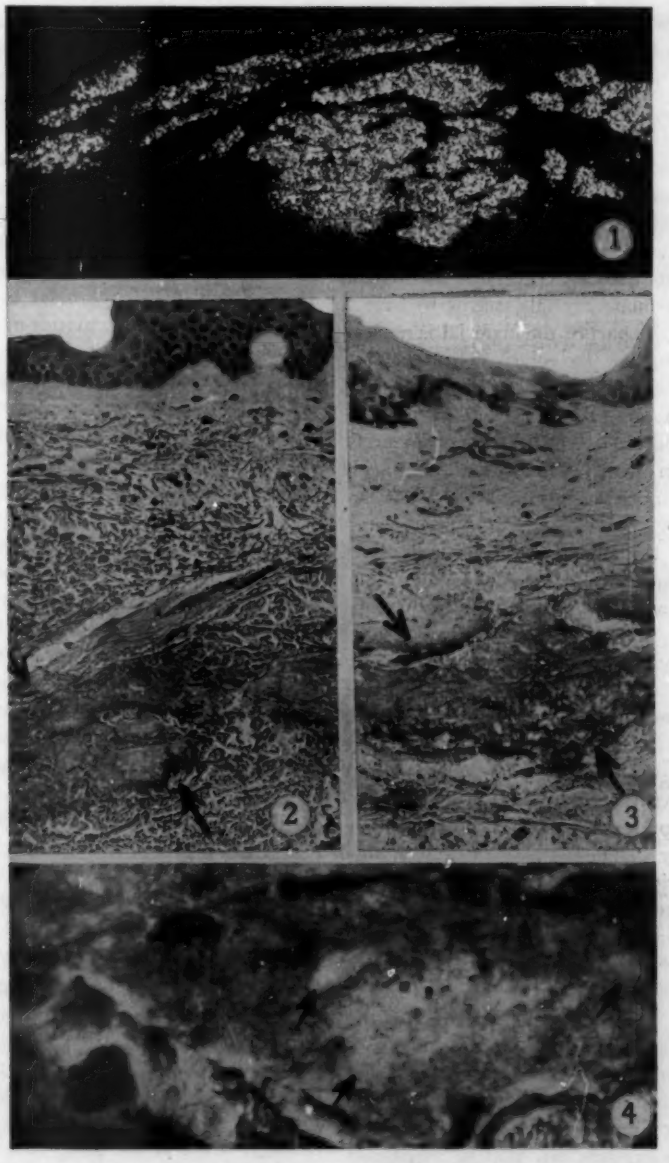
One-half hour after treatment the injection site was readily identifiable microscopically only by the birefringence of the hormone crystals. These crystals were located in the interstices of the dermal connective tissue. There was but little morphologic evidence either of the presence of the injection mass or of tissue reaction to it other than some mechanical separation of the connective tissue fibers.

Twenty-four hours after treatment the injection mass, previously detectable only with polarized light, became visible in hematoxylin-eosin preparations because of the formation of innumerable hematoxylinophilic particles among the hormone crystals (Figs. 1 and 2). These particles varied in size from several microns in diameter down to the limits of microscopic resolution. They also varied considerably with respect to their affinity for the hematoxylin. The depth of staining was not consistently related to particle size. The nuclei of the connective tissue cells in the injection mass were pyknotic, and many appeared to be fragmenting. The tissue fibers, however, appeared to be unchanged. Other than a moderate accumulation of leucocytes and macrophages at the free borders of the hematoxylinophilic mass, there was no evidence of an inflammatory reaction. When stained with acidified toluidine blue (Figs. 3 and 4) the nuclei, identifiable nuclear fragments, and many of the smaller particles in the injection mass were strongly orthochromatic, suggesting that they were composed, at least in part, of nucleic acids. Many of the smaller particles, however, were distinctly metachromatic, suggesting the presence of acid mucopolysaccharides. These conjectures were strengthened by several additional observations: (1) Deoxyribonucleic acid was demonstrable by the Feulgen reaction in many of the small basophilic particles, as well as in the intact nuclei and nuclear fragments; (2) after acid extraction of the tissue sections metachromasia disappeared and the intensity of the orthochromatic staining was reduced, and (3) many of the smaller particles were periodic acid-leucofuchsin-positive, the staining not being affected by prior digestion with malt diastase. The ninhydrin-leucofuchsin reaction was uniformly negative, indicating a low concentration of stainable protein-bound amino acid groups in the hematoxylinophilic particles. No free aldehyde was detectable by direct exposure to leucofuchsin without prior chemical treatment of the sections.

The microscopic findings in the paraffin-embedded tissues obtained 48 and 72 hours after treatment were essentially the same as those described above, except for a progressive decrease in the numbers of intact nuclei and large nuclear fragments within and near the periphery of the injection mass. Other than the continued presence of a moderate number of leucocytes and macrophages, principally at the periphery of the injection site, there was little or no further evidence of inflammatory reaction by the dermis.

Examination in polarized light of frozen sections of tissues removed ½ to 72 hours after treatment revealed that the injected steroid crystals could be visualized readily by their birefringence. No reduction in the amount of birefringent material could be detected during the period of observation. At 24 hours no lipid was found by any of the other methods employed, whereas at 48 and 72 hours scattered globules of sudanophilic lipid appeared in and at the periphery of the injection mass. For the most part the sudanophilic material was located in the macrophages, although a small proportion appeared to lie free in the interstitial spaces. Phospholipid was also present in the cytoplasm of the macrophages in the form of very small droplets, which could be seen only with the oil immersion lens. The Liebermann-Burchard and digitonin tests for cholesterol were uniformly negative throughout the period of observation.

It should be noted here that none of the histologic or histochemical alterations de-



Photomicrographs of histologic sections of normal human skin 24 hours after the intradermal injection of hydrocortisone acetate.

Fig. 1.—Masses of hormone crystals demonstrated by polarized light. × 80.

Fig. 2.—Hematoxylinophilic reaction mass (indicated by the arrows) formed among the hormone crystals. Hematoxylin-eosin stain; × 160.

Fig. 3.—Staining of the reaction mass (indicated by the arrows) by acidified toluidine blue. × 160.

Fig. 4.—Higher power of the reaction material stained with acidified toluidine blue, showing its granular nature. The light areas (indicated by the arrows) are "ghosts" of hormone crystals which have dissolved out during preparation of the slide. × 560.

scribed above resulted from the intradermal injection of the vehicle alone. In fact, the site of the injection of the vehicle was unidentifiable, microscopically.

Comment

The lesion which results from the local action of hydrocortisone in normal human dermis, i.e., the appearance and persistence of numerous basophilic granules among the injected hormone crystals, seems to have a dual origin. A part of the material is nuclear debris from the karyolysis accompanying death and disintegration of the connective tissue cells and, later, of infiltrating leucocytes. This is best seen in Feulgen preparations, in which deoxyribonucleic acid-containing nuclear fragments and smaller particles are found lying free and scattered throughout the site of the injection. Part of the basophilic material, on the other hand, appears to be composed of precipitated acid mucopolysaccharide. This is indicated by the presence of metachromatic as well as orthochromatic granules in the basophilic mass. In addition, many of the granules are stained with leucofuchsin subsequent to digestion with diastase and oxidation with periodic acid, further indicating the presence of polysaccharide other than glycogen. The histologic origin of this polysaccharide is not certain. The most likely sources are the ground substance of the dermis and the cytoplasm of the disintegrating cells. Since the cells constitute such a small portion of the total volume of the dermis relative to the volume of the ground substance, it seems probable that the metachromatic material is derived chiefly from the latter. The presence of sudanophilic lipids in and near the mass of hormone crystals can also be attributed to the breakdown of the dermal connective tissue cells and infiltrating leucocytes. No definitive explanation can be offered for the presence of the finely dispersed phospholipids in the cytoplasm of the macrophages. Although it is possible that these lipids may also have been derived from cellular disintegration, free phospholipid was not found in the interstitial spaces. It seems more likely, therefore, that the phospholipid is an integral component of the cytoplasm of the macrophages themselves.

The hydrocortisone-induced lesion in normal dermis appears to result from a paradoxical action of the hormone; that is, whereas the injected hydrocortisone causes destruction of dermal cells and ground substance, it also suppresses the inflammatory and reparative activities of the affected tissue. Hence the debris and degradation products remain in situ for long periods, owing to the slow rate of their removal in the presence of the injected hormone. The latter, in turn, remains in the dermis for long periods, owing to its suppression of the foreign body reaction and its relative insolubility in the tissue fluids. The fact that the lesion is limited to human skin is somewhat puzzling. All the biologic mechanisms which appear to contribute to its formation—cellular damage, changes in the ground substance, and suppression of tissue reaction to injury—have also been reported to occur in experimental animals. The answer to this problem must await further investigation.

In view of the present observations, it seems improbable that the basophilic masses formed in the skin lesions of patients injected intradermally with hydrocortisone are directly related to the beneficial therapeutic action of the hormone. This view is supported by the fact that, although cortisone alone induces similar basophilic masses in both normal and diseased skin, it has little or no therapeutic effect. For the present, at least, we must conclude that intradermally injected hydrocortisone produces local tissue damage, while at the same time it produces amelioration of preexisting disease processes through its suppression of the inflammatory reactivity of the dermal connective tissue.

Summary

Because of the therapeutic value of hydrocortisone in a variety of inflammatory diseases of the skin when administered topi-

cally, there is considerable current interest in the mode of the local action of the hormone in the dermis. The present study was undertaken to determine the morphologic effects of this hormone when injected into normal human skin, in the hope of shedding further light on the mechanism of its physiologic and therapeutic activity.

Microscopic examination of biopsy specimens removed from ½ to 72 hours after the intradermal injection of hydrocortisone acetate revealed the formation of a particulate mass of basophilic material at the site of the injection within 24 hours. From histologic and histochemical data, the lesion appeared to be composed in part of deoxyribonucleic acid derived from the nuclear debris of disintegrating dermal connective tissue cells and infiltrating leucocytes, and in part from precipitated acid mucopolysaccharide from the dermal ground substance. Free sudanophilic lipid, derived from the breakdown of dermal cells, was also present in the lesion. The connective tissue fibers did not appear to be affected during the period of observation. Despite the extensive morphologic damage, there was but minimal inflammatory reaction by the affected tissue.

In view of the previously known anti-inflammatory action of hydrocortisone and the present observations, it was concluded that the hormone acts by a paradoxical mechanism when used intradermally as a therapeutic agent. That is, it produces local tissue damage and yet inhibits the development of an inflammatory reaction to the damage it has caused. At the same time, the hormone suppresses the inflammatory process already present as the result of the disease agent previously affecting the skin.

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Multiple Dissecting Aneurysms of the Aorta

A Case Report

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The occurrence of multiple dissecting aneurysms of the aorta has been a distinct rarity. The first reported case was that of Shennan,[1] who in 1932 described the presence of four separate dissecting aortic aneurysms in a 64-year-old man. The same author,[2] in reviewing the world literature on dissecting aneurysms of the aorta, described 5 cases from a total of 300 in which multiple dissecting aneurysms occurred. Of these, three cases exhibited two dissecting aneurysms each, one case had three, and one case had four. Bay,[3] in 1944, reported as an incidental autopsy finding the presence of two small separate dissecting aortic aneurysms in a 68-year-old man, who died of a myocardial infarction. Bauersfeld,[4] in 1947, in reporting 15 cases of dissecting aortic aneurysms, described 1 case in which two dissecting aneurysms were found, the one being old, and the other acute with rupture into the pericardium. Review of the literature has revealed only one additional case, that of Hunter and Lium,[5] who, in 1952, recorded a case exhibiting two dissecting aortic aneurysms, one estimated to be two and one-half months old and the other an acute one.

The case under discussion is remarkable in that there were six separate and distinct dissecting aneurysms of the aorta, of varying ages, found at autopsy.

Report of Case

The patient, a 68-year-old white man, was admitted to Vanderbilt University Hospital for the fifth time on Feb. 16, 1953, in a comatose state. There was a history of severe hypertension for at least 10 years previously, and several episodes of angina-like pain had occurred in recent years. Previous admissions, during the preceding six years, had been for suspected myocardial infarction on two occasions, once for bleeding duodenal ulcer, and once for what was suspected to be a dissecting aneurysm of the aorta. The last episode, which occurred four months before the final admission, was characterized by the sudden onset of severe "knife-like" pain in the midthoracic region, with radiation around into the flanks. The blood pressure at this time was 240/120. After several days of hospital care the patient was sent home at bed rest. Four days prior to his final admission he began complaining of dizziness. During the day prior to admission he became increasingly lethargic and vomited frequently, finally becoming comatose.

Physical Examination.—The patient was found to be an emaciated, elderly white man in a comatose state. Blood pressure was 160/90, temperature was 101 F, and the pulse was 160 per minute and totally irregular. Coarse moist rales were heard over both lung fields. The left border of cardiac dullness was in the sixth left intercostal space, in the anterior axillary line. The liver was palpable one fingerbreadth below the right costal margin. Physical examination was not otherwise remarkable.

Hospital Course.—Following admission the patient continued to have auricular fibrillation. He became partially responsive by the third hospital day, but following this he again became comatose. There was a continuous low-grade fever, the temperature averaging 101 F. A chest x-ray suggested the presence of pneumonia in the upper lobes of both lungs. He died quietly seven days after admission.

Autopsy Findings.—The autopsy, begun two hours post mortem, revealed the following findings of interest: multiple dissecting aneurysms of the aorta (to be described in detail below); hemopericardium secondary to rupture of a dissecting aneurysm of the ascending aorta; marked generalized arteriosclerosis and arteriolar sclerosis; cardiac hypertrophy, predominantly left ventricular; chronic duodenal ulcer; bilateral hydrothorax; acute passive congestion of the liver, and bilateral organizing pneumonitis.

The source of the hemopericardium was found to be a 3 mm. ragged tear located in the center

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of an area of swelling on the right lateral aspect of the ascending aorta, 3 cm. above the aortic valve. Viewed externally, the aorta exhibited three additional areas of localized swelling, one on the left posterolateral aspect of the arch immediately distal to the left subclavian artery; one on the left lateral aspect of the descending thoracic aorta 10 cm. above the aortic hiatus of the diaphragm, and a third one projecting outward from the left posterolateral aspect of the descending thoracic and abdominal aorta. This last area of swelling, which was the largest, measured 6 cm. in longitudinal length, 2.5 cm. in width, and projected outward 2 cm. When the aorta was opened, six intimal defects were noted, all in relation to the externally visible areas of swelling of the aortic wall. At 1 cm. above the aortic valve there was a 3.5 cm. ragged intimal tear, which communicated with the external aortic rent. The area of swelling of the aorta at this point was the result of the intramural accumulation of fresh partially clotted blood. Five additional cleft-like intimal defects were present, each being filled with organizing clotted blood. The first and smallest of these was located 0.2 cm. beyond the most distal portion of the above ruptured aortic aneurysm. Its intimal orifice measured 1.0 cm. transversely and 0.5 cm. longitudinally. The four additional intimal defects were similar in appearance and somewhat larger. Two of these were located in the distal portion of the aortic arch, one in the descending thoracic aorta, 13.0 cm. above the aortic hiatus of the diaphragm, and the last one, which was the largest, was located on the left posterolateral wall of the descending thoracic aorta, 4.5 cm. above the celiac artery. Cut sections through the aorta at the level of each of these intimal tears showed the presence of old, clotted, and organizing blood, which had dissected the aortic wall for varying distances. The dissection extended either proximally or distally, or in both directions from the site of intimal tear. The largest area of dissection, which involved the descending thoracic and abdominal aorta, extended a total of 6.0 cm. in length and measured 2.0 cm. in greatest thickness.

Heart and aorta showing the openings into the intima of six dissecting aneurysms. Aneurysms 1 and 3 are marked by arrows.

Examination of other portions of the aorta revealed the presence of moderately severe atherosclerosis. In spite of the numerous areas of the aorta involved by the processes of dissection, only a few minor branches of the aorta were compromised or obstructed by their presence. These included the first left bronchial artery and the fifth, sixth, seventh, and ninth right and left intercostal arteries.

Microscopic Findings

Sections from each of the areas of dissecting aneurysm and from uninvolved areas were stained with hematoxylin and eosin, Mallory's aniline blue and Weigert's elastic tissue stain. Varying degrees of degenerative medial changes were seen in all of the sections, in addition to varying degrees of atheromatous degeneration of the intima. There was a marked decrease in the amount of elastic tissue in the media. The number of smooth muscle fibers in the media was also greatly diminished, and many remaining muscle fibers were split. In a few areas clefts filled with pale amorphous debris were present between collagenous lamellae. Occasional groups of endothelial-lined, cavernous vascular channels were present in the media adjacent to areas which had been involved by dissection. The location of the plane of dissection within the media varied. One aneurysm split the external elastic lamina. Two of the lesions were in the inner media near the internal elastic lamina, and three of them were in the midportion of the media. In the adventitia most of the vasa vasorum

showed varying degrees of proliferative thickening of their walls, and occasional small artery being almost obliterated.

Comment

This case presents an unusual example of the complications which may follow the occurrence of degeneration of the media of the aorta. The histopathology of this lesion has been well described by many authors * in the past; however, the pathogenesis remains obscure. The exact incidence of multiple dissecting aneurysms of the aorta is unknown; however, their rarity can probably be assumed, on the basis of the small number which have thus far been reported. The patient described in this report developed and successfully recovered from a total of five dissecting aneurysms over an indefinite period of time, probably, however, covering many months or years. Only with the sixth dissecting aneurysm did external rupture of the aorta occur, with resultant hemopericardium and death. It is well documented [10] that dissecting aneurysms of the aorta need not rupture externally but may rupture internally into the aortic lumen, as occurred here, or they may remain intramural in the form of a hematoma within the aorta wall. It is interesting that this patient, in spite of the occurrence of several separate dissecting aortic aneurysms, had no obstruction of any of the major branches of the aorta, this fact undoubtedly constituting an important factor in his survival.

* References 2 and 6 through 10.

Summary

A case is described which presents the occurrence of six separate dissecting aneurysms of the aorta in a 68-year-old white man. Five of these lesions, which occurred at various levels in the thoracic and abdominal aorta, were old and communicated with the aortic lumen. The sixth, however, was acute and had ruptured into the pericardium with resultant hemopericardium.

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Periarteritis Nodosa in an American Deer

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The older literature of periarteritis nodosa contains two queer and rather tantalizing reports. One is the claim of Harris and Friedrichs * that they reproduced the disease in animals by inoculation of a Berkefeld N filtrate from human lesions, thus proving, to themselves at least, that periarteritis nodosa was caused by a filtrable virus. This report has never been substantiated; but so far as I know no one has ever reported having tried the Harris and Friedrichs experiments and failed. The second is the report of a kind of epidemic of periarteritis nodosa in deer, which was thought to prove its contagiousness. The present incomplete, and perhaps inconclusive, report is an attempt to shed light on the second of our two mysteries. I have, unfortunately, no access to the kind of material which would settle this question. Perhaps this paper may serve to interest those who have the material to examine it with the periarteritis nodosa question in mind.

In 1906 Lüpke,[3] a pathologist working in the veterinary medical school of Stuttgart (southern Germany), reported to the German Medical Society that, although the total reported human cases of the rare disease described by Kussmaul and Meier 40 years earlier numbered by that time only 17, he had himself seen a larger number, 23, in a single herd of Axis deer. Furthermore, he said that the Württemberg wild-life authorities had known of the disease for over 100 years and that it was responsible for the loss of up to 100 head in that herd. In the year 1890, of the 12 deaths in the herd, 6 were from periarteritis nodosa. In 1877, before Lüpke's observations, there had been an even larger "epidemic."

Lüpke wrote to the director of the zoological gardens in Calcutta, which boasted a large herd of Axis deer and which was near the source from which the European Axis deer originally came. He was told that the disease had never been seen there and that the herd was well. This, by the way, is the only justification I could find for the statement made in recent articles that the disease is not found in other deer.

Even if there had not been later histologic confirmation,[4] there would be little doubt that Lüpke's report concerned a pathologic condition we would today accept as periarteritis nodosa. The vascular changes were limited to the greater circulation, and the pulmonary circulation was spared. Mostly the aorta and larger branches were involved, and the posterior part of the body more than the chest, neck, and head. Spleen, liver, and kidneys were always involved, the heart often. Less commonly adrenals, ovaries, uterus, and testes were affected. The brain showed only slight thickening of arteries. The essential lesion was a nodular or a diffuse thickening along the course of arteries. Narrowing of the lumens up to complete closure was present, and there were outpouchings (aneurysms?) of various forms and sizes. Characteristically, many stages of the process were present in a single animal. Catarrhal gastroenteritis was never absent, and pulmonary infection was frequent. The author makes the point that these "complications" sometimes so masked the vascular lesions that the latter had to be looked for with care. No parasites were found.

The conclusions drawn from these findings were (1) that periarteritis nodosa is a specific infectious disease probably due

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* References 1-2.

to a filtrable virus, (2) that it originated not in the tropical homeland of Axis deer but in central Europe, and (3) that, since deer have no syphilis and they do have periarteritis nodosa, human periarteritis nodosa is not due to syphilis. The logic of the last set of propositions is shaky, but the conclusion has been confirmed on other grounds.

In 1909 A. Jaeger [4] had the opportunity to study Lüpke's material histologically. His descriptions leave no doubt that the lesions would be acceptable today as those of classical periarteritis nodosa. Search was made in the sections at that time for bacteria and protozoa, but none were found. Jaeger reported that in the meantime the "epidemic" was apparently over and that the herd, which had sunk to as low as 8 to 12 head, had by then increased to 27. "This exquisitely enzootic character of the course of the disease," he said, "shows with certainty that for the etiology of the process an organized infectious inciting agent must be assumed," by which he means, no doubt, a living agent, bacterium or virus.

No cases of periarteritis nodosa have been reported in deer since 1909. Reports on spontaneous cases in other animals are few and far between. Most of them were summarized by Baló [5] in 1924, who reported a case in a fox terrier (photographs very convincing) and mentioned cases in calves and in a swine. No epidemics are reported.

Present Study

This brings us down to the official hunting season of late fall 1955, when a white-tailed deer was shot in New York State. The hunter did not state whether this deer was unusually easy to bag; this is not the kind of information one is likely to elicit easily. At any rate, while preparing the carcass, he found an unusually large hock on one leg, which he cut out, together with surrounding soft tissues, and gave to a surgeon friend, who presented it to me. An x-ray of the specimen showed an old, largely unhealed fracture with callus, sequestrum, and pseudoarthrosis. Particles of metal indicated a previous gunshot wound and made it probable, the penalties for hunting deer out of season being what they are, that the lesion was almost exactly one year old.

Gross examination of the sawed section showed the old healing fracture, markedly eburnated sequestrum, and exuberant callus. There was no pus. Numerous sections were taken not only from near the fracture but from soft tissues at the periphery of the specimen. The gross condition of the arteries was not noted, and, unfortunately, through an error, the gross specimen was discarded before the sections were examined and the unusual findings noted.

Sections from near the bone showed new formation of bone, considerable proliferating connective tissue, and islands of intense

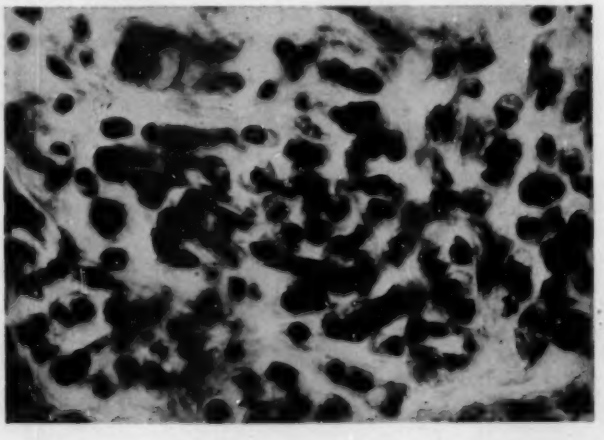

Fig. 1.—Interstitial inflammatory infiltrations made up predominantly of plasma cells. Hematoxylin and eosin; reduced to 2/3 of mag. × 220.

plasmocytic infiltration (Fig. 1). The relationship of plasma cells and plasmocytosis to immune processes, globulins, and amyloidosis is today in the forefront of attention.[6] Their role in hypersensitivity and collagen diseases has received particular attention.† The finding of a predominantly plasmocellular inflammatory infiltration in these sections is therefore probably not without interest and importance for the arterial changes which were so prominent.

Many arteries near the bone, and even more in the periphery of the specimen, showed intense arteritis. The walls were thickened, and all layers were infiltrated by lymphocytes and plasma cells, together with a few neutrophile leucocytes (Figs. 2 and 3). The media was disorganized; the intima was thickened; elastic tissue was completely or almost completely destroyed. Thrombosis was present in only a few vessels. An aneurysmal dilatation was seen only once (Fig. 4). About one-fourth of the lesions showed fibrinoid necrosis in the subintima or the media or both; the necrosis was nowhere massive.

Some weeks after the examination described above a portion of muscle (about half a pound) from one haunch was made available from the carcass, which had been deep-frozen. Many sections through this

† References 6-7.

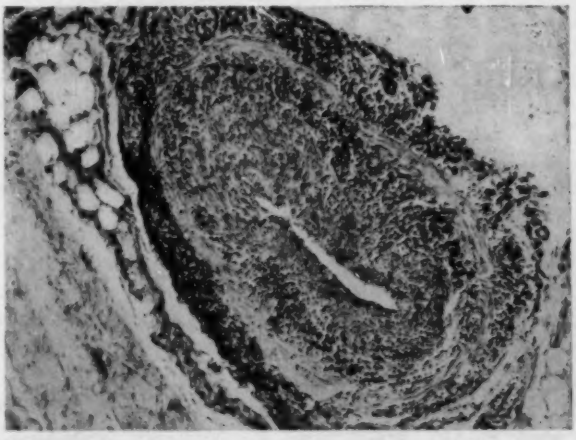

Fig. 2.—Typical periarteritis nodosa lesion. Subintimal necrosis. Lesions such as this showed almost no remaining elastic tissue. Hematoxylin and eosin; reduced to 2/3 of mag. × 140.

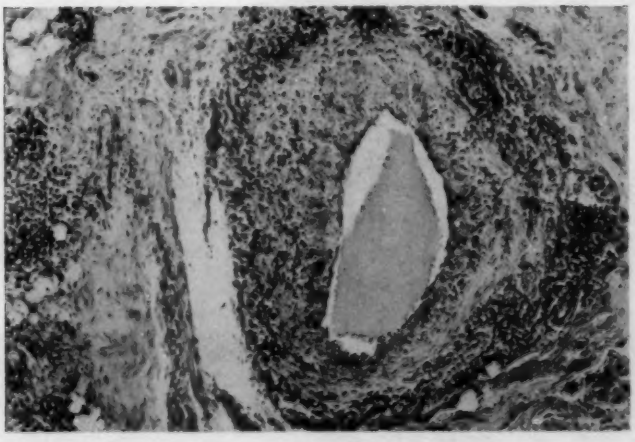

Fig. 3.—Periarteritis nodosa lesion. More necrosis and somewhat less inflammatory infiltration. Hematoxylin and eosin; reduced to 65% of mag. × 140.

Fig. 4.—Beginning aneurysmal dilatation at left. Hematoxylin and eosin; reduced to 3/5 of mag. × 140.

failed to disclose lesions of the blood vessels. The muscle showed a mild infection with Sarcocystis.

The lesions of periarteritis nodosa vary considerably from case to case and from vessel to vessel in the same case. In a study soon to be completed, I shall try to show that about the most constant finding in classical cases is injury to the elastic tissue. I mentioned above that the involved vessels had no remaining elastic tissue, or almost none. In addition, when other vessels were studied which showed no inflammatory infiltration, a change in the internal elastic membrane could be seen (Figs. 5 and 6). This consisted of disappearance of segments of the elastic membrane. Almost always, the regions of absent elastica were marked by a cushion-like swelling and proliferation of the intima, which was absent from the regions of intact elastic tissue; this conjunction of missing elastica and intimal proliferation makes it unlikely that the failure of the elastica to stain in areas is an artifact or a postmortem change.

Comment

Much of the discussion about periarteritis nodosa today centers about the problem of whether classical periarteritis nodosa is different from other forms of "necrotizing angiitis" or whether they are essentially similar processes. Zeek and her associates‡

‡ References 8-9.

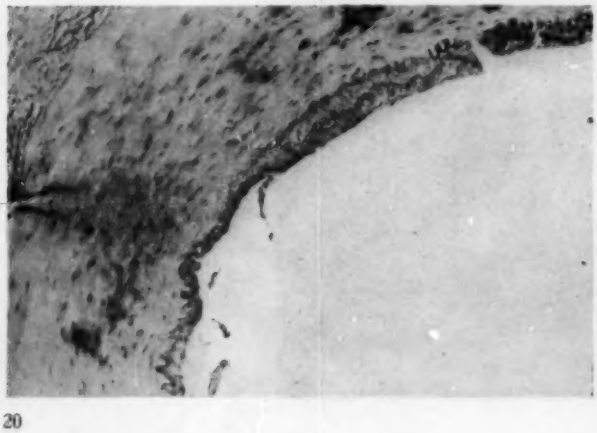

Fig. 5.—Artery with no cellular infiltration. Areas of destroyed elastic tissue and, in the same places, proliferation of the intima. Verhoeff's elastic tissue stain; reduced to 2/3 of mag. × 140.

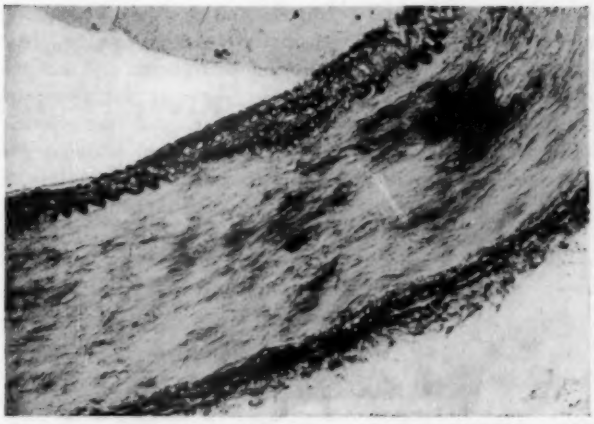

Fig. 6.—Smaller areas of absent elastic tissue but with the same intimal proliferation. Verhoeff's elastic tissue stain; reduced to 2/3 of mag. × 140.

have argued forcefully for separation, and would like especially to separate "hypersensitivity angiitis" from classical periarteritis nodosa, whereas I § have tried to indicate reasons that too complete a separation may have disadvantages. The real reason for this nosologic argument is, of course, a concern not so much for classification as for the etiologic implications. If classical periarteritis nodosa and hypersensitivity arteritis are separate and distinct conditions with no overlap, there is no reason to have to admit hypersensitivity mechanisms as competent stimuli in the pathogenesis of periarteritis nodosa. Perhaps we should continue the search for the living agent which is the unique cause of the disease; perhaps we should repeat and extend the experiments of Harris and Friedrichs. If, on the other hand, the various forms of necrotizing angiitis form a single group the members of which fade into each other and overlap, then it is probable that mechanisms which operate in one member of the group may operate in the others. This means that, since hypersensitivity mechanisms have been fairly well proved to be competent stimuli in one of the forms, they may also be such in the classical periarteritis nodosa. My point of view ‖ has been that all members of the group of necrotizing angiitis may sometimes be the result of hypersensitivity mechanisms but that all may also result from other mechanisms.

Obviously, the report of an epidemic form in deer is weighty evidence in favor of the uniqueness of classical periarteritis nodosa. Is there any way to fit the fact of this epidemic into the hypothesis that periarteritis nodosa is often the result of allergic mechanisms? There is; and my little observation on one deer leg, while it proves nothing, points the way to such an explanation.

Both animal experiments and human observation related to hypersensitivity show marked individual variation. There are "allergic" and "nonallergic" animals and humans, and heredity has something to do with the difference. Even in the Rich ¶ experiment for the induction of periarteritis nodosa there is considerable variability,[18] and I can personally testify to the exasperating frequency with which some rabbits refuse to develop lesions. The "allergic" animal or human is one who will generally become sensitive to a wide variety of agents if the proper conditions are present. Now, suppose that deer as a family (New York deer are not the same genus as the Axis deer of India), or certain strains among them, are unusually likely to develop vascular localization whenever they become hyper-

§ References 6 and 10.

‖ References 6 and 10

¶ References 11-12.

sensitive. Suppose that even with well-known infections (gastroenteritis or pulmonary infection, as reported by Lüpke) they developed nodular arteritis. Suppose, even, that this could happen when an endemic disease affected a herd. Then the arterial localization would appear to be endemic, without the necessity for periarteritis nodosa to be due to a special and separate agent. In such case other deer will develop similar lesions with other causes, maybe after infected gunshot wounds.

There remains the problem of the ending of the epidemic. If deer are especially susceptible to arterial lesions after a variety of infections, why no periarteritis nodosa since 1909? It will be remembered that the disease lasted about 100 years, during which an original herd of over 100 was reduced to 8 to 12. It is entirely probable that this number represents the 8% to 12% which, like the rabbits in the hypersensitivity experiments, do not develop arteritis. They are the only ones left to pass down genes, and these genes no longer include those which determine a tendency to hypersensitivity. The disease has been wiped out not by the disappearance of a living agent but by the process of natural selection.

The truth of this hypothesis will be known when enough deer with enough kinds of disease will have been examined with these facts in mind. It is to be hoped that those who have the opportunity for studies of this kind will undertake them. It is hoped that these will include the examination of many arteries, visceral as well as local.

Summary

1. An observation is reported of typical periarteritis nodosa lesions in the soft tissues around a year-old gunshot wound and fracture in a New York State deer.

2. The facts are reviewed concerning an old report of an epidemic of periarteritis nodosa in a herd of deer in southern Germany.

3. A hypothesis is constructed wherein it is supposed that the frequency of periarteritis nodosa in deer is caused by a special predisposition, probably hereditary, to hypersensitivity reactions or to their arterial localization or to both; and that the apparent epidemic form is the result of some epidemic disease, not periarteritis nodosa, which is a competent stimulus for production of periarteritis nodosa lesions through hypersensitivity mechanisms.

4. It is urged that those who have an opportunity to study deer tissues keep this hypothesis in mind and make the observations necessary to prove or disprove it.

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Experimental Production of Thyroiditis

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Very few attempts have been made to produce thyroiditis in experimental animals. The first successful attempt was made by McCarrison in 1927. To white rats he fed a diet deficient in vitamins but adequate in iodine. In 25% of the animals in his first series and in 10% of his later experiments he obtained lymphocytic infiltration and fibrosis of the thyroid.

Bastanie, in 1937, fed 10 rats white bread and gave as drinking water a 1% solution of iodine. In five rats he obtained lymphocytic infiltration in the thyroid. Of eight guinea pigs fed bread, beets, and hay, Bastanie obtained inflammatory changes in the thyroids of two. Five other guinea pigs received the same diet for 120 days and thyrotropic hormone (TSH) afterward. These five animals showed epithelial degeneration and lymphocytic infiltration of their thyroids. Bastanie regarded avitaminosis as the probable cause of these experimental goiters.

In recent times, Clausen undertook the experimental production of thyroiditis. In 1953 he fed young rats a well-balanced diet and gave 0.1% thiouracil as drinking water. After 300 days 6 of the 24 animals showed degeneration of acinar epithelium, absence of colloid, lymphocytic infiltration, and fibrosis. In 1954 Clausen fed a balanced diet to 35 male rats and gave 0.2% thiouracil as drinking water. At the end of 24 months six of the animals showed loss of colloid, lymphocytic infiltration, and marked fibrosis in their thyroid. Clausen attributed these changes to an excess of thyrotropic hormone, accepting my working hypothesis (1938).

If one considers that in autopsy material 16% of normal thyroids show focal areas of thyroiditis, these older experiments are certainly not impressive. Recent attempts to produce thyroiditis by radioactive iodine were much more successful.

Goldberg and co-workers injected I^{131} intraperitoneally into white rats and observed marked inflammation of the thyroid. Within 12 hours the epithelial cells became swollen and vacuolated. After 24 hours edema, polymorphonuclear infiltration, and follicular disintegration occurred. Within six to eight months the thyroid tissue was replaced by hyaline fibrous tissue.

Gorbman observed infiltration of the thyroid with lymphocytes and polymorphonuclear leucocytes in mice 48 hours after injections of I^{131}. After 120 days there was only a shrunken fibrous band in place of the thyroid.

In 1952 Maloof, Dobyns, and Vickery injected I^{131} into male rats. They obtained, after two days, a loss of follicular architecture, desquamation of follicle cells, and infiltration of the stroma with monocytes and lymphocytes. At the end of one year the thyroids were replaced by dense scar tissue. In contrast to the lower percentage of positive results in other experiments with vitain deficiency or thiouracil, I^{131} seems to produce nonspecific, nonsuppurative inflammation in 100% of the experimental animals.

We studied the effect of I^{131} on the thyroid of white rats and guinea pigs to elucidate the mechanism of the inflammatory reaction and, if possible, to determine whether the response to I^{131} differs from spontaneous "idiopathic" thyroiditis in man.

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Material and Methods

Male adult rats of the Sprague-Dawley strain, weighing between 300 and 400 gm. each, were used throughout. They were maintained on a diet of Purina Chow and tap water, to which the animals had access throughout the experimental periods.

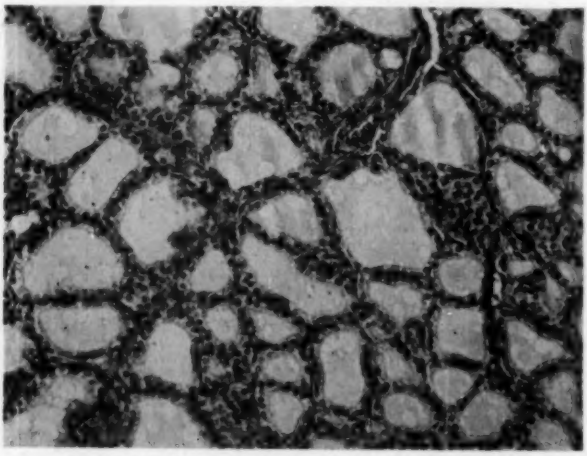

Fig. 1.—Normal thyroid gland of adult male white rat (Purina Chow and tap water). Scanty interstitial tissue. × 140.

Each rat received a single intraperitoneal injection of 50, 88, 100, or 100μc of I^{131} to which no carrier had been added. The rats were kept in metal cages with screen bottoms to prevent access to feces. At various intervals after the I^{131} injection the rats were anesthetized with pentobarbital (Nembutal) and exsanguinated. The soft tissues of the neck anterior to the prevertebral fascia were removed *in toto* and fixed in 10% formalin for histologic examination. Paraffin sections 6μ to 8μ thick were stained with hematoxylin and eosin.

Ten rats were used as controls. Forty-five animals received I^{131}; nineteen received TSH, and ten received propylthiouracil. In Table 1 the number of animals in the different experimental groups is listed. In all, 83 animals were used.

TABLE 1.—*Experimental Groups of Animals, Drugs and Duration of Experiments*

Group	No. of Animals	Drugs and Dosage	Duration of Experiment
1	10	Controls (Purina Chow and tap water)	
2	9	TSH, 1 unit daily for 5 days	2 wk. to 280 days
3	10	Propylthiouracil, 10 mg. daily for 5 days	2 wk. to 280 days
4	10	TSH and propylthiouracil same as in 2 and 3	2 wk. to 280 days
5	14	I^{131} 50 μc to 100 μc	24 hr. to 2 wk.
6	2	I^{131} 300 μc.	48 hr.
7	17	I^{131} 1 mc.	24 hr. to 40 wk.
8	11	I^{131} 1 mc., and TSH 1 unit, daily for 5 days	2 wk. to 40 wk.

Results

The data presented in Table 2 are based on the examination of the thyroids of 83 rats.

1. Rats injected with 50μc to 100μc of I^{131} showed no evidence of damage to the thyroid after a period of 24 hours to 2 weeks. Forty-eight hours after injection of 300 mc. of I^{131} the follicle cells were slightly swollen and had vacuolated cytoplasm and the colloid showed many vacuoles. There was no interstitial edema or inflammatory cell reaction, and no macrophages were seen.

2. In rats injected with 1 mc. of I^{131}, the thyroids showed capsular and interstitial

TABLE 2.—*Inflammatory Reactions in Thyroid Glands of Different Experimental Groups*

Group	Drugs and Dosage	No. of Animals	Reaction in Thyroid
1	Controls	10	
2	TSH, 1 unit daily	9	None
3	Propylthiouracil 10 mg. daily, 5 days	10	None
4	TSH & propylthiouracil same dosage as 2 & 3	10	None
5	I^{131}, 50 μc to 100μc	14	None
6	I^{131}, 300 μc.	2	Vacuolation of cells in two
7	I^{131}, 1 mc.	17	Severe thyroiditis
8	I^{131}, 1 mc., and TSH, 1 unit daily, for 5 days	11	Severe thyroiditis

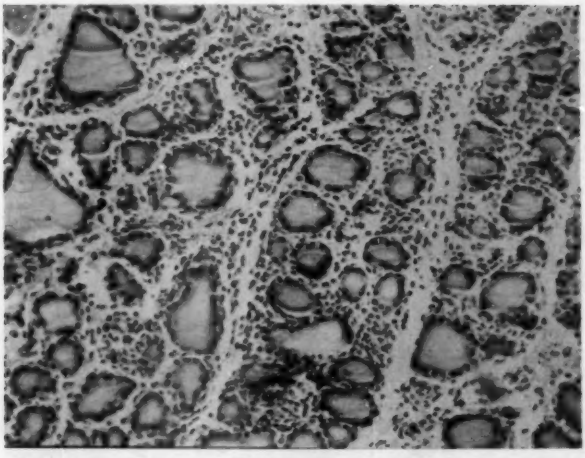

Fig. 2.—Thyroid gland, 24 hours after injection of 1 mc. of I^{131}. Disruption of some thyroid follicles. Beginning infiltration with lymphocytes and monocytes.

edema after 24 hours. This resulted in wide separation of the follicles. The glands were infiltrated with monocytes, lymphocytes, and very few neutrophilic leucocytes. The colloid was dense and well stained. There was no vacuolation of colloid noticed. Most epithelial cells were cuboid, well stained, and without evidence of damage. Occasionally disintegrated follicles were seen in the center of the lobes. The lumen was empty, the follicle wall collapsed, and the cells were vacuolated. Monocytes were seen in the wall and in the lumens of these follicles. Many capillaries were dilated, but there was no swelling of the endothelial cells. In the capsule, between thyroid tissue and muscle, many monocytes and lymphocytes were noticed.

Forty-eight hours after injection of 1 mc. of I^{131} the follicles were widely separated by edema and cellular exudate. Of the inflammatory exudate, three-fourths was made up of monocytes and one-fourth of lymphocytes. Most follicles were of small size and contained thick, well-stained colloid without vacuoles. The cells were high-cuboid and oxyphilic. A few were light-stained and contained vacuoles. There were many nuclear fragments in the stroma. The capillaries were dilated, and there were small hemorrhages. In the walls of a few follicles monocytes were noticed between the epithelial cells.

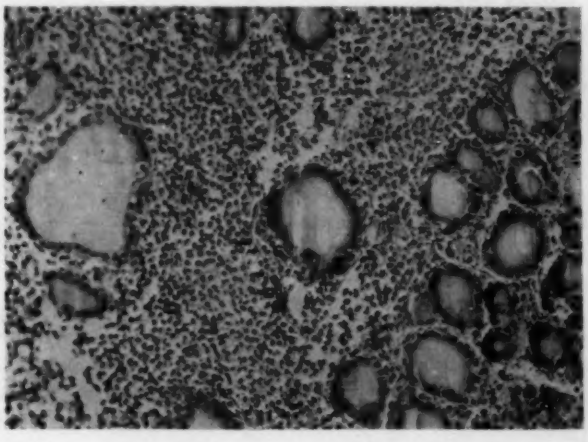

Fig. 3.—Ninety-six hours after injection of 1 mc. of I^{131}. Follicles separated by marked interstitial edema and infiltration with monocytes and lymphocytes.

Seventy-two hours after I^{131} injection the exudate was much less extensive. There was complete disorganization of follicular structure in the center of the glands. Most follicles were shrunken, collapsed, and had defects in the wall. The cells varied in size and were poorly oriented. Most of the colloid had disappeared. There were few monocytes and lymphocytes between their follicles, and groups of epithelial cells were seen without follicular structure. Many epithelial cells were vacuolated and had very large and dark nuclei. Monocytes prevailed markedly over lymphocytes, and occasionally intrafollicular colloid contained macrophages.

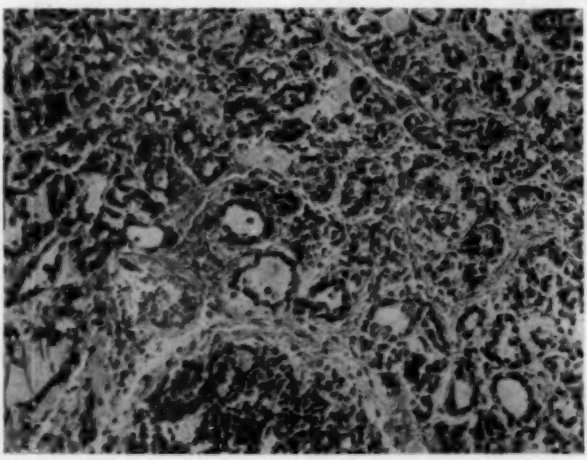

Fig. 4.—One week after injection of 1 mc. of I^{131}. Most of colloid removed by monocytes. Disorderly follicular structures. Cessation of inflammatory reaction.

After eight days, thyroid follicles were preserved only in the isthmus and in the periphery. The center formed a hyaline mass with occasional single cells. Narrow tubules with dark, degenerated cells were seen in the periphery. Few follicles contained colloid. In a few follicles which had no colloid, monocytes were present in the lumen. There were few inflammatory cells in the glands. Large cells with iron pigment were noticed in the stroma.

After two weeks, very few follicles could be recognized. The hyaline stroma contained solid or alveolar groups of large, vacuolated cells. Only an occasional follicle contained colloid. No inflammatory cells were seen in the central portions of the gland.

After four weeks, most of the glands were completely fibrosed. The scar tissue contained brown pigment and only a few lymphocytes, monocytes, and occasionally some polymorphonuclear leucocytes. In one pole were degenerated small follicles, incompletely surrounded by large, vacuolated cells and without colloid.

After 40 weeks, near the well-preserved parathyroid gland, dense scar tissue and few small follicles were seen as only remnants of the thyroid tissue, and the cells were large, oxyphilic, and granulated. Some had giant nuclei; the colloid was unstained in some, thick and deep-red in other follicles. In the dense scar tissue, cells containing brown granules and a few lymphocytes were almost the only cells. In one thyroid, perivascular infiltration with lymphocytes and monocytes was seen.

It can be concluded from the examination of the thyroid glands of 83 rats that radioiodine in adequate doses will produce, without exception, nonsuppurative thyroiditis.

The earliest changes which we observed consisted of swelling and vacuolation of the follicle cells. Their degeneration and necrosis of certain cells led to disruption of follicles. The third stage of thyroiditis

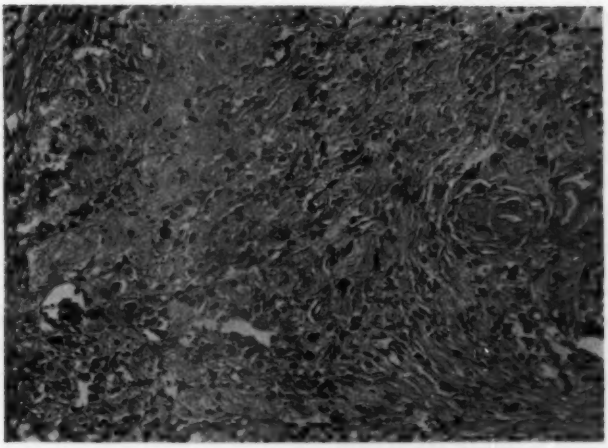

Fig. 5.—Four weeks after injection of 1 mc. of I^{131}. Fibrosis with few scattered thyroid cells and lymphocytes replacing thyroid gland.

manifested itself by intensive infiltration of the stroma with monocytes and lymphocytes, associated with edema. Two weeks later fibroblasts appeared, and reparation with fibrosis commenced.

After several months, epithelial proliferation was noticed, especially in the peripheral portions of the gland. Large, oxyphilic cells with nuclei of varying size formed small follicles or solid glands. As pointed out by Goldberg, these cells resembled the oxyphilic cells of Hashimoto's disease.

Comment

Nonsuppurative thyroiditis has been explained as the result of a great variety of etiologic factors. The two authors who recently made a thorough literary review of chronic thyroiditis (Hazard and Goetsch) came to the conclusion that the etiology of thyroiditis is uncertain. The experimental production of nonsuppurative thyroiditis has, until now, contributed nothing to clarify the etiology of thyroiditis. The results of the older experiments with vitamin defi-

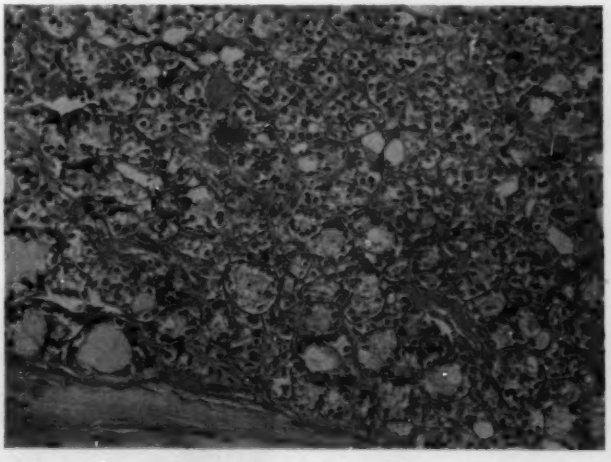

Fig. 6.—Ten weeks after injection of 1 mc. of I^{131}. Regenerative proliferation of large, oxyphilic thyroid cells.

ciency and thiouracil (McCarrison and Bastanie) were so inconsistent that they appear meaningless.

Radioiodine in adequate doses appears to procure positive results, which attain a percentage of 100. Our experiments on male white rats gave as unequivocal results as those by Goldberg, Gorbman, and Maloof. While, of course, radioiodine cannot play a role in the spontaneous "idiopathic" thyroiditis in man, the histological study of thyroiditis of animals treated with radioiodine may be valuable to elucidate the mechanism of thyroiditis.

Although physical agents, radium, roentgen rays, radioisotopes, are well known as inflammatory agents, the thyroiditis following radioiodine injection is much more intensive than inflammatory reactions to ionizing radiation in other organs. Goldberg and co-workers believe that injury to the thyroid epithelium is the underlying cause of the inflammatory response. We would attribute more importance to follicular disruption and exposure of colloid to the vascular interstitial tissue. In our experiments, edema and cellular infiltration of the thyroid did not occur until—due to necrosis of some thyroid cells—the follicles became disrupted.

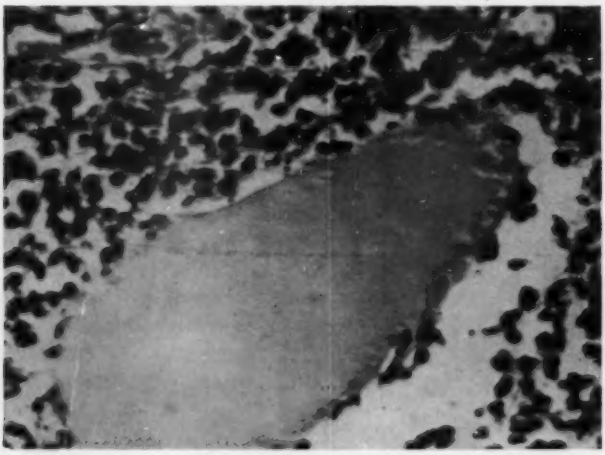

Fig. 7.—Leucocytic reaction in subcutaneous tissue of guinea pig four days after implantation of colloid.

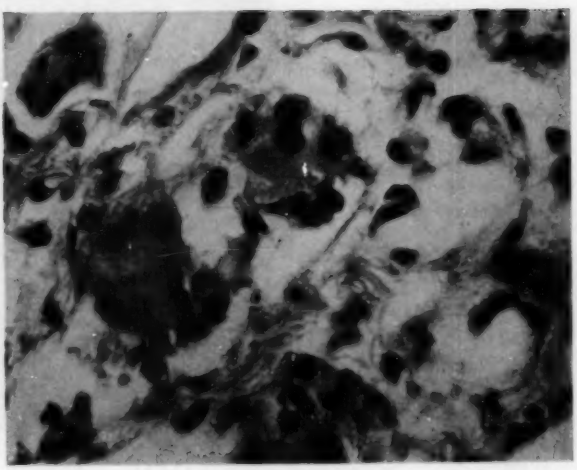

Fig. 8.—Monocytes, giant cells, and lymphocytes in subcutaneous tissue of guinea pig 13 days after implantation of colloid.

Our view is supported by Ferguson's observations that thyroid colloid injected into the subcutaneous tissue of guinea pigs provokes a marked local inflammation. We could confirm his observations by implanting slices of colloid-rich glands into the subcutaneous tissue of guinea pigs. Figures 7 and 8 show lymphocytes, monocytes, and multinuclear giant cells attracted by the colloid.

TABLE 3.—*Implantation of Thyroid Colloid into Subcutaneous Tissue of Guinea Pigs, Duration, and Histological Findings*

Implant	Duration, Days	Histological Findings
1	4	Acute cellulitis, mostly by polymorphonuclear leucocytes
2	6	Infiltration with polymorphonuclear leucocytes, monocytes, multinuclear giant cells
3	13	Monocytes, giant cells, fibroblasts
4	13	Monocytes, fibroblasts, multinuclear giant cells
5	13	Monocytes, giant cells, fibroblasts, eosinophiles
6	12	Lymphocytes, eosinophiles, giant cells, monocytes
7	12	Pseudotubercles, consisting of monocytes, lymphocytes, and giant cells

We, therefore, believe that, from the etiologic standpoint, thyroiditis following radioiodine is comparable to pancreatitis, of which, in spite of a great variety of etiologic factors, extravasation into the tissue spaces of pancreatic enzymes is the immediate cause. Thyroiditis would then belong to the group of inflammations caused by endogenous secretory products of specialized tissues (Forbus).

Conclusions

Very few attempts have been made to produce thyroiditis in experimental animals. The results following a vitamin-deficient diet or administration of thiouracil have been uncertain.

In experiments of 83 white male rats we could confirm Goldberg, Gorbman, and Maloof's observations that radioiodine, in adequate doses, is a most powerful etiologic factor of thyroiditis.

The disruption of follicles precede the edema and cellular infiltration of the thyroid tissue in animals injected with radioiodine.

It seems very probable that exposure of colloid, following disruption of thyroid follicles, to vascular stroma of the glands plays an important role in attracting monocytes and lymphocytes.

Implantation of thyroid colloid into subcutaneous tissue of guinea pigs provones a marked local inflammation.

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Regeneration of the Fundic Mucosa in Rats

Effect of Estrone and of Castration

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The factors governing regeneration of the mucosa of the digestive tract are poorly understood. This applies to physiologic as well as to pathologic regeneration. Some authors consider that mucosal regeneration is retarded in chronic peptic ulcer,* and the importance of studies of possible factors which may retard or promote healing in this disorder has been stressed.[1] In gastric cancer abnormal regeneration is often discussed as a pathogenetic factor, especially in cases in which gradual transition from peptic ulcer or chronic gastritis is claimed.[2] The importance of mucosal regeneration in gastric surgery is also apparent.

Certain observations may point to sex hormones as possible regulators of regeneration of the gastric mucosa. Peptic ulcer displays a sex-linked character, with a pronounced predominance in males, and favorable results have been reported of the use of estrogens for ulcer patients.[4] A promoting effect of female sex hormone on development of gastric ulcer has been claimed in pylorus-ligated rats.[5] Attention has also been called to the age distribution and sex discrepancy in gastric cancer, and an endocrine basis has been suggested.[4] The possible relationship between the androgen-estrogen ratio and gastric carcinoma has been pointed out.[4] So far, however, there is no convincing evidence of hormonal factors in this disease.[6]

The present study aims to investigate the regeneration of experimental defects in the fundic mucosa in rats and the effect of castration and of estrogen administration on this.

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* References 1-2.

Materials and Methods

The defect was produced by the excision of a wedge-shaped area of the anterior fundic wall, measuring about 2×6 mm. The defect was then closed, serosa to serosa, with three or four sutures. At the end of the observation period the animal was killed by ether and autopsy performed immediately afterward. This method has the advantage that at the time of operation sections of the gastric wall are available for histologic control. The stomach was opened along the greater curvature and stretched out on a cork plate. The specimens were fixed in 10% formalin. The animals were kept on the standard diet of our laboratory. The age of the rats ranged from 2 to 18 months at the time of operation.

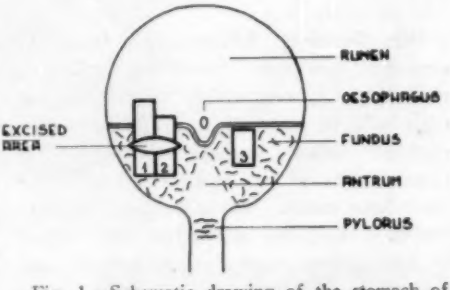
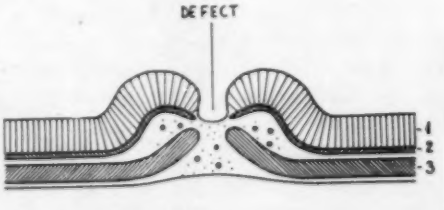


Fig. 1.—Schematic drawing of the stomach of the rat, opened along the greater curvature and stretched out. It shows the areas examined microscopically (*1, 2, 3*).



1. MUCOSA.
2. MUSCULARIS MUCOSAE.
3. MUSCULARIS.

Fig. 2.—Schematic drawing of the microscopic section through the fundic wall at the site of excision.

Figure 1 demonstrates schematically the various parts of the stomach, the excised area, and the sections taken out for microscopic examination. The operation leaves a defect, as shown in Figure 2. From each stomach an average of 18 sections, 12 from the defect, were examined microscopically. The sections were stained with hematoxylin-eosin and mucicarmine, and occasionally also with Van Gieson's method.

Two hundred forty animals were divided into three main groups: (1) control, (2) castrated, and (3) estrogen-treated animals. Each group contained 40 males and 40 females. All animals were operated on in exactly the same way. Castration was carried out seven days before the gastric operation. In the third group the animals got 100γ of estrone daily, injected subcutaneously in oil solution, from the seventh day before the operation. The injections were continued until the 30th day after operation, when the animals were not killed earlier. Twelve animals died of complications, mainly hemorrhage.

In order to register the changes in the healing mucosa, groups of eight animals—four males and four females—were killed at two-day intervals from the 5th to the 15th day, and thereafter on the 21st, 30th, 60th, and 180th day after the operation.

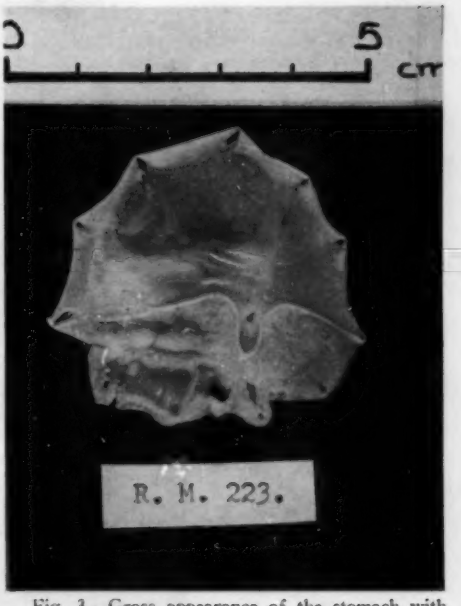


Fig. 3.—Gross appearance of the stomach with the folds on each side of the defect. Compare with the drawing in Figure 1. Control group, seven days postoperatively.

Results

Healing of the Defect.—As mentioned, the operation leaves a defect, which will diminish owing to muscular contraction, the edges inclining toward each other. The defect has the characteristics of an ulcer over which the regenerating epithelium migrates. The gross picture is seen in Figure 3. The folds on the sides of the defect are most prominent during the first days, but the gross appearance changes very little at the different stages.

A single layer of epithelium grows from the edges of the defect (Fig. 4). The most advanced cells are flat; the others are cuboidal or tall columnar. The columnar epithelium invaginates, forming small pits, which develop into new glands (Fig. 5). In three weeks, the new mucosa approaches normal appearance (Fig. 6); the cells, however, are still undifferentiated. Parietal and chief cells are not seen. The regeneration and the differentiation continue, and after 180 days it is often difficult to distinguish the new mucosa from the surroundings (Fig. 7). Parietal cells are clearly seen at this stage.

Diastasis.—In 33 animals there was a large gap in the mucosa because of a diastasis between the muscular layers due to suture insufficiency (Fig. 8). As a matter of fact, it will take more time to cover these extensive defects. In these cases the base was covered by a thick necrotic layer, which seemed to delay healing.

Surface Epithelium.—Several microscopic criteria were used in order to estimate the findings. Some of these are summarized in the Table.

No particular difference was observed among the three main groups with regard to epithelial covering of the defects. A slight difference in the groups might be noticed during the first five to nine days, healing in the control group being a little more retarded than in the two other groups. From the 11th day onward no difference was found. The majority of animals in all groups showed complete healing of the defect from the 13th day onward. In 4

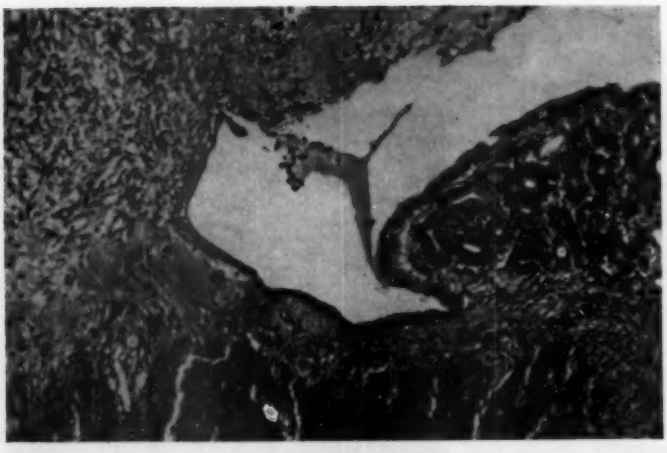


Fig. 4. — Epithelium growing over the defect from the edges. Control group five days postoperatively. Reduced to 92% of mag. × 125.

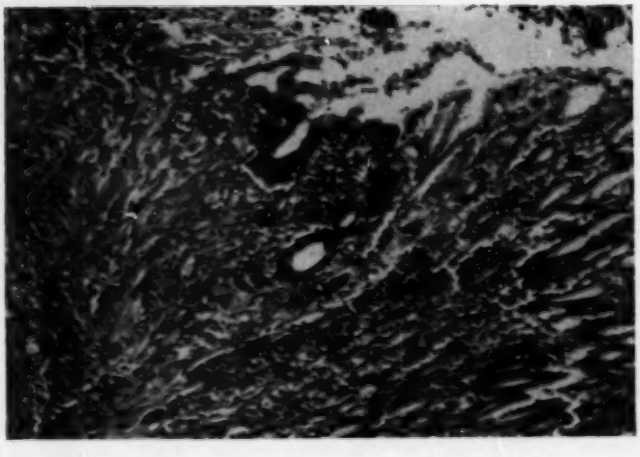


Fig. 5. — Invagination of epithelium and formation of new glands. Control group, seven days postoperatively. Reduced to 92% of mag. × 125.

Fig. 6.—The new mucosa approaching normal appearance. Estrogen-treated group, 21 days postoperatively. Reduced to 92% of mag. × 18.

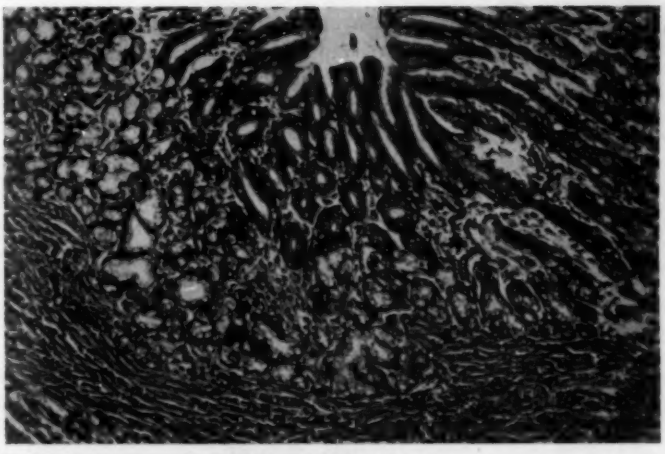


Fig. 7.—The new mucosa hardly distinguishable from the surrounding mucosa. Parietal cells are seen. Castrated group, 180 days postoperatively. Reduced to 92% of mag. × 125.

Fig. 8.—An artifact, showing the large diastasis between the muscular layers and the extensive defect. Estrogen-treated group, 60 days postoperatively. Reduced to 92% of mag. × 18.

Microscopic Criteria

		CONTROLS										CASTRATION										ESTROGEN-INJ									
NUMBER OF ANIMALS		6	6	6	6	6	6	6	6	7	7	7	6	6	6	7	6	6	6	6		7	6	6	6	6	6	6	7	6	
DAYS AFTER OPERATION		5	7	9	11	13	15	21	30	60	180	5	7	9	11	13	15	21	30	60	180	5	7	9	11	13	15	21	30	60	180
COVERING OF DEFECT	< ½	6	6	6	4							7	3	3	1							6	5	2	1						
	½				1	2		2		1*	1*			5	4	3		1*	3*			1	2	2	3		1*		2*		
	< 2				2	2	1		1*							3	2			2*	1*				3	2				1*	
	2					1*		6	5	6	6	7			1	1	6	6	5	6	7	8	1	1	2	6	7	6	8	4	6	
NEW GLANDS	0	6	3		1							7	3	2								7	4								
	+			5	8	7		2			1			5	6	4	1							2	7	2						
	++						6	8	3	3	1					4	5	4	5	2					1	6	5	4	2	1		
	+++								5	5	5					2	3	2	4	4	2						3	4	6	7	5	2
	++++										7						1	2	4	6										2	4
PARIETAL CELLS		0	0	0	0	0	0	0	3	5	7	0	0	0	0	0	0	2	7	7	6	0	0	0	0	0	0	0	7	5	6
CHIEF CELLS		0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	1	1	4	6	0	0	0	0	0	0	0	1	2	2
DILATED GLANDS		0	3	6	6	6	7	5	5	6	3	0	4	6	6	7	7	7	4	6	0	0	6	7	6	5	6	6	3	5	2
NECROSIS/DEFECTS*		0/6	1/6	2/6	1/6	3/6	2/6	3/6	1/6	1/6	0/6	7/7	3/6	5/6	1/6	3/7	1/6	3/6	1/6	1/6	0/6	0/6	3/6	2/6	1/6	0/6	2/6	1/6	0/6	3/6	1/6

* Large diastasis
* Necrotic layer on the base of the defect / Number of preserved defects

of the 22 animals killed on the 13th day—2 belonging to the controls and 2 to the castrated group—a defect was still present, as also in 5 animals of the 23 killed on the 15th day—3 from the controls and 1 from each of the two other groups. Two of the latter five animals showed the aforementioned diastasis. Of the 91 animals killed on the 21st day and later, diastasis was found in all 12 showing incomplete cover-

ing. The main factor delaying healing in these cases seemed to be suture insufficiency, rather than the experimental conditions. Practically the same percentage of delayed healing caused by large diastasis was found before the 13th day.

There seems therefore to be a remarkable agreement among the three main groups with regard to regeneration of surface epithelium, since covering was found in all groups from approximately the 14th day, excluding artifacts.

No difference between the two sexes was found in the three main groups as to the covering of the defect.

Glands.—No significant difference was demonstrated in the three main groups with regard to development of new glands, either during the first days or later. On the seventh day a few new glands were formed, and thereafter a steady increase in number occurred. The scattering was about the same in all groups. Mitosis was frequently seen in the new glands (Fig. 9). On the 13th day several new glands were formed (Fig. 10), but parietal and chief cells were not found until the 21st day. Parietal cells were found in all animals killed after 180 days.

Dilated glands were present at the edges of the defect in nearly all animals, being mostly pronounced from the 9th to the 21st day.

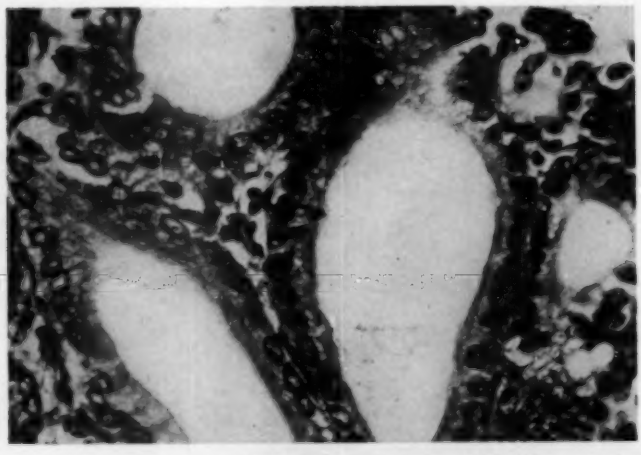


Fig. 9.—Mitosis in the new glands. Control group, nine days postoperatively. Reduced to 92% of mag. × 500.

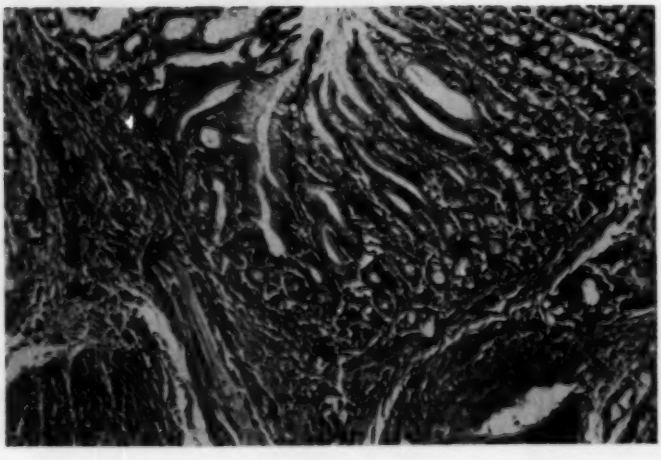


Fig. 10.—Several new glands formed on the 13th day. Castrated group, 13 days postoperatively. Reduced to 92% of mag. × 125.

In the majority of cases there was a necrotic layer over the granulation tissue in the floor of the defect. This applies to the first days, but particularly to the period from the 15th day on, all persisting defects being covered by a necrotic layer.

Comment

The present investigation shows that, under the experimental conditions employed, neither castration nor estrogen administration has any demonstrable effect on the healing of experimental defects of the stomach in rats of either sex. Thus, the reported favorable and unfavorable influence † on the healing of gastric ulcers has not been confirmed.

There is no agreement regarding the general effect of the estrogens.‡ Estrogenic effect on gastric functions is theoretically possible, since estrogens may influence endocrine organs, such as the pituitary gland and others,§ and since the stomach has been regarded as an endocrine organ,[17] owing to its own hormone production.

The investigations confirm earlier observations ‖ that repair of experimental defects occurs, with a gradual transformation from undifferentiated to highly differentiated cells in the gastric mucosa.

Further investigations are in progress with regard to the possible effects of other hormones. Emphasis must be laid on the connective tissue and on the gastric secretion, at least in the experiments showing a difference in the regenerating capacity.

Summary

The regeneration of experimental defects in the fundic mucosa in rats and the effect of castration and of estrogen administration on this regeneration have been studied. Two hundred forty animals, of both sexes, were divided into three main groups: (1) control, (2) castrated, and (3) estrogen-treated animals. The rats were killed at two-day intervals from the 5th to the 15th day, and thereafter on the 21st, 30th, 60th, and 180th day after the operation.

No significant difference was found in the various groups with regard to epithelial covering of the defect and development of new glands. Excluding artifacts, all groups showed complete covering of the defect from approximately the 14th day. No difference between the two sexes was recorded.

† References 4-5.
‡ References 7-14.
§ References 12, 15, 16.
‖ References 2, 18, 19.

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Adenoacanthoma of the Stomach

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The occurrence of epithelial neoplasms showing both glandular and pavement histologic components limited to the pyloric end of the stomach is one of interest, not only because of the rarity of the neoplasm but because of the problem of its histogenesis. Herxheimer [1] described the neoplasm as adenocancroid, and it has been successively known as adenosquamous carcinoma (Rabson [2]), Malpighian epithelioma (Gauthier-Villars [3]), and polymorphous epithelioma (Oberling [4]). Currently, the accepted term is adenoacanthoma (Pasternack [5]).

Wood,[6] in 1953, collected seven authenic cases from the literature and added two of his own. Since that time 10 additional cases have been described.*

This neoplasm has been described as occurring in the cardioesophageal region of the stomach as well. While a somewhat different explanation may be offered for the histogenesis of adenoacanthoma in this region, it is of interest to compare the relative frequency of this tumor in the two portions of the stomach. In 1943 Wood reviewed the literature on stomach tumors which showed epidermoid characteristics and found a total of 19 such tumors. Seven were adenoacanthomas limited to the region of the pylorus; three were instances of adenoacanthoma not limited to the pyloric region, and nine were pure epidermoid carcinomas. With these he also included five cases of squamous-cell carcinoma and one case of adenoacanthoma which he considered as arising in the esophagus rather than the stomach. In 1948, McPeak and Warren [8] reviewed 276 consecutively resected carcinomas of the stomach and found 10 adenoacanthomas, 8 of which occurred at the cardioesophageal junction and 2 in the pyloric region. Tumors which occur in this region may be reported with neoplasms of either the cardia or the esophagus, introducing statistical inaccuracies. Since it is often impossible, even at histologic examination, to determine the site of origin of this group of tumors, it would appear wise to group these tumors apart from gastric and esophageal tumors.[14]

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Report of a Case

A 70-year-old white man was admitted to the U. S. Public Health Service Hospital with the chief complaints of a dull ache in the right upper quadrant of the abdomen following meals, and exertional dyspnea. Five years prior to this admission, he had been treated in this hospital for squamous-cell carcinoma of the lower lip. One year prior to admission, his case was worked up on the cardiovascular service. In the course of this study, a left bundle-branch block and achlorhydria were found.

The significant physical findings were a filling defect in the greater curvature in the pyloric and prepyloric portion of the stomach and a left bundle-branch block. Accordingly, surgical exploration was done, and it was immediately apparent that the patient had an inoperable gastric carcinoma located in the pyloric segment of the stomach with metastatic spread to the omentum and liver. No surgical intervention apart from biopsy was attempted. The patient died suddenly the morning of the first postoperative day, after several brief episodes of fecal vomiting.

Autopsy

The cause of death was mesenteric thrombosis with infarction of the terminal ileum and peritonitis. Severe cerebral and coronary atherosclerosis was also present. The stomach was empty. In the posterior wall, 6 cm. from the pyloric ring, was a 4 cm. ulcerated area penetrating deeply into the stomach wall, but not perforating it. The ulcer was marginated by an elevated indurated area, extending 2 to 3 cm. out from the ulcer itself. On the cut surface grayish-white tumor tissue

was seen replacing all layers of the stomach wall. The serosal surfaces of the stomach, pylorus, and first part of the duodenum were studded with coalescing tumor nodules. The serosal surfaces of the liver and gall bladder were covered with tumor nodules, which varied from 1 mm. to 1 cm. in diameter and in many areas were coalescent.

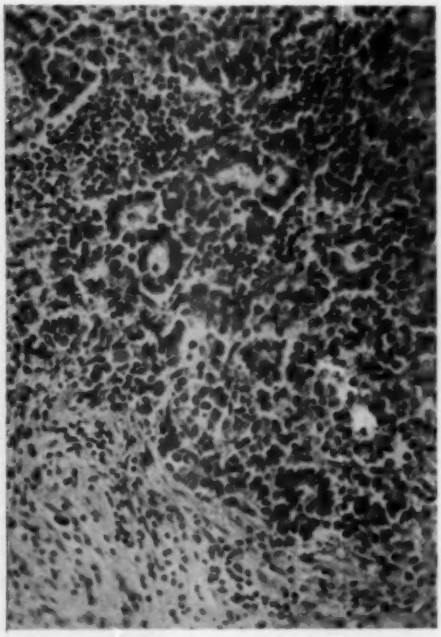

Fig. 1.—Section of primary tumor. Here, the neoplasm is entirely adenocarcinoma. Hematoxylin and eosin; reduced to 59% of mag. × 200.

Microscopic Examination

Sections through both the ulcer and its margins had essentially the same picture. The mucosa and muscularis were being replaced by neoplastic cells, which were widely invading the tissue lymphatics and were infiltrating the subadjacent serosa and fat. The neoplastic cells differentiated to give two histologic patterns. In some areas the neoplasm was composed of oval or polygonal cells, often having scanty cytoplasm and large, often eccentrically placed nuclei, which possessed prominent nucleoli (Fig. 1). These cells tended to form both acini and ducts, but were also seen proliferating in sheets of cells. In other areas the neoplasm was composed of large polygonal

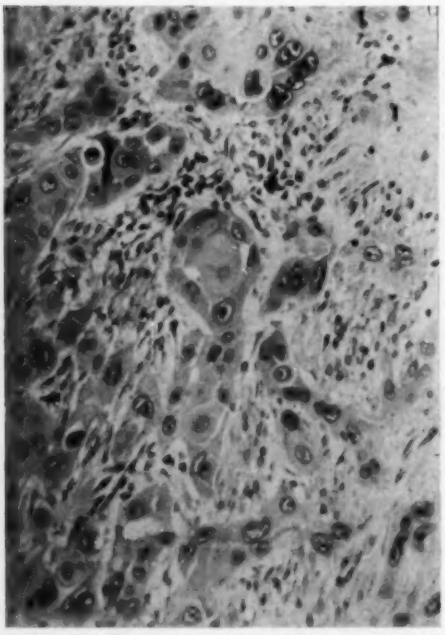

Fig. 2.—Section of primary tumor; neoplastic squamous-cell component. Hematoxylin and eosin; reduced to 59% of mag. × 200.

cells having varying amounts of amphophilic cytoplasm and hyperchromatic, often bizarre, nuclei, and proliferating to form nests and cords of cells (Fig. 2). These cells showed squamous differentiation, with well-formed intercellular bridges and abundant keratin formation. In irregular areas of both histologic cell types, necrosis was a prominent feature.

The neoplastic invasion of the omentum, regional lymph nodes, fat, serosal surfaces, and liver was entirely by cells showing squamous differentiation.

Comment

The occurrence of a pavement type of epithelium where this does not normally occur has provoked many hypotheses which attempt to explain the development of adenoacanthoma.

Herxheimer believed that the neoplastic squamous and glandular elements took their origin from undifferentiated embryonic cells, rather than from more mature ec-

topic cells. Lubarsch [15] suggested the possibility of true metaplasia of normal mucosal cells preceding, or occurring simultaneously with, the development of the neoplasm. Oberling and Wolf explained one of their cases as metasplasia of adult cells, and the other as congenital heterotopy. Krompecher [16] believed that nonembryonic undifferentiated basal cells normally present in the gastric mucosa gave origin to the neoplastic glandular and pavement components. Stewart and Lorenz [17] concluded from their experimental work, in which true adenoacanthomas were produced in the forestomach and the pyloric stomach of mice by the injection of methylcholanthrene, that, under carcinogenic influence, a normal glandular mucosa could redifferentiate into glandular and squamous neoplastic components. Wood. The case of Strassmann presented the belief that adenoacanthomas develop as the result of direct stimulation of the gastric glandular mucosa by a carcinogen, and that, once neoplasia is initiated, varying degrees of redifferentiation may develop, manifested by the presence of both glandular and squamous cells. Indeed, at the present time, the majority of writers accept the hypothesis of direct neoplastic stimulation of undifferentiated basal cells in the gastric mucosa as the essential factor. However, it should be noted in passing that Casanova Diaz [18] has offered the so-called gastric semisquamous epithelial layer of Duran-Jorda as the source of squamous epithelium occurring in all mucous membranes.

It is of interest to note the cell type in the metastases. The character of the primary neoplasm in the case of Lubarsch was recognized only after squamous cells were found in the tumor metastases. The squamous-cell component was limited to the primary tumor in the case of Oberling and Wolf; the metastases were entirely glandular. The same histologic picture obtained in the case of Pasternack. The metastases were mixed in the two cases reported by Wood. The case of Strassman presented metastases composed of pure squamous-cell carcinoma, and the case being reported also showed only the squamous-cell component in the metastatic neoplasm.

Careful histologic study of many tumors will often give some clue to the histogenesis, but this does not seem to be true of the adenoacanthoma. The primary tumor, in the case being discussed, was limited to the pyloric and prepyloric region of the stomach.

This precludes the squamous-cell component of the neoplasm from having taken its origin in congenital heterotopies of esophageal mucosa, for these have never been reported in this anatomic area of the stomach. As judged from the sections taken, the primary tumor was almost equally composed of squamous-cell carcinoma and adenocarcinoma, and both these showed approximately the same degree of differentiation. This suggests that, once the neoplastic process was set in motion, redifferentiation took place, with the formation of both glandular and squamous neoplastic cells, and reaffirms Wood's belief that the lesion is carcinomatous from the beginning.

Summary

A case of adenoacanthoma occurring in a 70-year-old white man is reported. The tumor occurred in the posterior wall of the stomach in the pyloric and prepyloric region and had metastasized to the mesentery, liver, and serosal surfaces of the stomach, duodenum, and gall bladder. Histologically, the primary tumor was composed of neoplastic cells which showed both squamous and glandular differentiation. The metastases were composed only of squamous-cell carcinoma.

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Gonadal Dysgenesis and Associated Anomalies (Turner's Syndrome)

Report of a Case with Autopsy Findings

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In recent years the term "ovarian agenesis" has been applied to a condition apparently first described in detail by Morgagni in 1761.* However, prior to 1938, when Turner described seven young women with "infantilism, congenital webbed neck, and cubitus valgus,"[3] there were few publications directing attention to the coexisting malformations which are now well known. Some of the early studies concentrated on the genital abnormalities,[4] and others elaborated on a complex of anomalies, including webbing of the neck ("status Bonnevie-Ullrich").[5] Subsequently many publications † have demonstrated the principal features of congenital gonadal deficiency and have shown the frequent association of a number of developmental anomalies. The condition is said to be "not uncommon," although sometimes confused with the dwarfism and related manifestations of pituitary hypofunction.

On the basis of the peculiar malformations in relation to sexual immaturity, the original conception of the syndrome has been extended in many directions. There are even a few reports of "Turner's syndrome in the male,"[10] as well as a number of cases of "ovarian agenesis with mild virilization."[11] Nevertheless, most of the patients have been short, sexually immature, but definitely feminine persons. The condition is of practical interest, since the administration of estrogens to these young women results in the desired maturation of feminine secondary sexual characteristics.[12] However, there are reasons to question the propriety of the term "ovarian agenesis." Recent studies of the epidermal nuclei have shown that most of these patients have chromatin patterns like those of males and presumably were males at conception.[13] Previous work ‡ had demonstrated that animals of some species when castrated at the proper stage in fetal life would develop into sterile and immature but definite "females," regardless of the sexual genotype.

Aside from the study of nuclear patterns there seems to be no means of determining the sexual genotype of these patients. Possibly those with virilization are genotypic males with functioning testicular elements or perhaps a greater production of (or susceptibility to) other androgens. Be that as it may, nearly all of the published cases have been irrevocably feminine, and there has been no published evidence that the desired effects of estrogen administration are modified by the genotype. The rare cases of the male phenotype who have infantilism, webbing of the neck, seem to be just as definitely masculine and theoretically should all be genotypic males.

The associated anomalies have considerable significance, since they may suggest the diagnosis and treatment of the hypogonadism and prompt a search for remediable

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† References 6 through 9.

‡ References 13 and 14.

deformities, such as coarctation of the aorta.[15] They are also of interest in the study of the etiology and pathogenesis of the condition. One theory[9] is that the primary anomalies are a consequence of injury to certain regions or metabolic processes of the embroyo at a critical stage, possibly from a maternal viral infection, hormonal imbalance, or other adverse condition. An inherited genetic defect seems unlikely from the rarity of reports of a familial incidence.[16] Turner § has seen the syndrome in identical twins, but this does not necessarily indicate a hereditary relationship. Indeed, injury to the genes of the ovum, sperm, or zygote seems more likely. The strange spectrum of seemingly unrelated but frequently coexisting anomalies might indicate a juxtaposition of potential organizing factors, such as is found in the arrangement of the genes in chromosomes.

The sexual immaturity, osteoporosis, delayed epiphyseal closure, and some of the other abnormalities are attributed to gonadal deficiency. The administration of estrogens is effective in most respects. However, prevention of growth retardation in cases diagnosed in childhood remains a problem. Since postnatal castration ordinarily results in increased height, the short stature of virtually all of these patients is paradoxical and usually is attributed to a genetic abnormality.

Thus it would seem that a better understanding of the condition might be of value clinically as well as academically. The following case, while characteristic of Turner's syndrome, presents several additional features of possible significance.

Report of Case

The patient, a 50-year-old white woman, according to some relatives was in some way abnormal from birth, but her siblings attributed her physical and mental retardation to an injury in early childhood. No developmental abnormalities were known in any relatives. The family noticed that she had a short neck with a low hairline posteriorly and prominent folds of skin bilaterally. Earaches were said to be frequent in childhood, and the patient was partially deaf. Her feet were proportional to her stature (size 1½ shoes), but her hands were relatively large (size 6½ gloves). At about age 30 her few remaining teeth were extracted because of marked pyorrhea. She often had a "cold" and cough, but was otherwise considered healthy. She attended school for years but never learned to read or write anything except her signature. She was described as cheerful and industrious, regularly assisting in household chores.

In the last two years of her life the patient lost much of her child-like hyperactivity, possibly in relation to the illness and death of her father.

A year before death she fainted once and was hospitalized three weeks for anemia, pulmonary rales, ascites, pedal edema, diarrhea, and enlargement of the heart and liver. Occult blood in the foul-smelling stools led to the removal of two small benign rectal polyps. Diagnostic studies included a Kepler-Power-Robinson water test (inconclusive; index A value of 29) and attempts to do a Thorn test. However, the fasting circulating eosinophile count on two occasions was below 44 per cubic millimeter. Transfusions were given, and she was discharged much improved.

Six months before death she had a mild febrile illness ("flu"). Subsequently she developed insomnia, became petulant and moody, and had bizarre complaints and "screaming spells." She was committed to a mental hospital, where she was considered to have the mentality of a 9-year-old, with poor insight and judgment. No explanation of the marked change in behavior was established. The spinal fluid protein, serology, and gold curve were normal. Roentgenograms were said to show moderate pulmonary emphysema and generalized osteoporosis, with "lytic lesions" of the skull which suggested multiple myeloma. No Bence Jones protein was found in the urine, but the total serum protein was reported as 6.95 gm/100 ml., with an A/G ratio of 3.2/3.75. The blood urea nitrogen was said to be 39 mg/100 ml.

Two months before death the patient was transferred to the University of Oklahoma Hospitals for further study.

Physical Examination. — Physical examination revealed a poorly nourished, sexually immature "dwarf" (height 53 in. [135 cm.], weight 55 lb. [25 kg.], whose face appeared much older than her 50 years. Webbing of the neck and cubitus valgus were described. Although subcutaneous tissue was very scanty, wrinkling of the skin was pronounced only on the face. There was a moderate thoracic kyphoscoliosis. The scalp hair was coarse and dry. Coarse brown hairs over the lips and chin were more evident than usual for a woman of this age. Several elevated hairy nevi were present about the nose and lips. The breasts and genitalia were considered to be "prepubertal."

§ Turner, H. H.: Personal communication.

The ribs were very prominent, with beading at the chondral junctions. Further examination was unsatisfactory due to lack of cooperation.

Laboratory Data.—Laboratory data included a hemoglobin of 10.5 gm/100 ml., with a hematocrit reading of 34%. White blood cell counts ranged from 7000 to 9200 per cubic millimeter, with neutrophilia and a shift to the left. Urinalyses and determinations of serum calcium and phosphorus were within the normal range. Serum cholesterol was 144 mg/100 ml. Total serum protein was 6.5 gm/100 ml., with the A/G ratio 4.2/2.5. The blood urea nitrogen was normal on two occasions. The circulating eosinophile count was reported as 9.3 per cubic millimeter, fasting. The response to hypoglycemia induced by intravenous insulin was considered normal. The radioactive iodine uptake of the thyroid was 20% at 24 hours (normal 15% to 40%). The urinary 17-ketosteroid excretion on successive days was reported as 4.4 and 3.1 mg. daily. (Normal adult women excrete 6 to 14 mg. daily, while women without adrenal cortical function excrete less than 1 mg. daily.) Urinary gonadotropin excretion was more than 52 mouse units daily; no assays were made at higher dilutions. Roentgenograms showed generalized osteoporosis and a marked irregularity of the iliac crests at the sites of closure of the epiphyses.

On the basis of the above data it was believed that multiple myeloma was excluded and the diagnosis of "ovarian agenesis" was substantiated. Although further studies were desired, proper facilities for care of disturbed patients were not available, and she was returned to the mental hospital. The administration of diethylstilbestrol was suggested, but no hormones were given. She died unexpectedly in July, 1954, following a brief illness with fever and diarrhea.

Autopsy Findings.—The cause of death was determined to be bronchopneumonia. External examination confirmed the clinical descriptions. Hair of the upper lip and chin was coarse, brown, and up to 1 cm. in length. Axillary hair was entirely absent. The very sparse pubic hair was straight and measured up to 2 cm. in length, although less than a dozen hairs exceeded 1 cm.

The heart weighed 145 gm. There was more than the usual amount of epicardial adipose tissue, which, however, exhibited marked serous atrophy, as did the scanty adipose tissue elsewhere. The coronary arteries were very tortuous and on section were found to have foci of atherosclerosis of slight or moderate degree. The aortic valve had only two cusps, but these were well formed and delicate. There was no stenosis or insufficiency. Both coronary arteries arose from the aortic sinus of Valsalva nearest the pulmonary artery. The foramen ovale was not patent.

There was a slight but definite coarctation of the aorta 0.8 cm. distal to the ligamentum arteriosum. The aortic lumen measured 4.5 cm. in circumference here, in contrast to 6.0 cm. at the aortic valve and 7.0 cm. in the dilatation distal to the narrow constricting ridge. The aortic arch was elongated and unusual in that the three major arteries were widely separated.

There were moderate fibrous pleural adhesions over the lateral aspects of both lungs. The left lung had no lobar fissure, and the right had only a horizontal fissure 3 cm. in length and 0.2 cm. in depth. There were no interlobar fibrous tissue septa to suggest obliteration of the fissures by pleuritis. The pulmonary parenchyma had a nodularity compatible with acute bronchopneumonia. Slight tubular bronchiectasis was most evident in the inferior part of the left lung.

The spleen appeared large in proportion to the body size, weighing 200 gm. It was unusually short, broad, and thick. The capsule was thickened and contained multiple small yellow nodules.

The capsule of the liver was also slightly thickened. Cut surfaces suggested minimal portal cirrhosis and moderate fatty change. The gall bladder was proportionately large, but the biliary system was not otherwise remarkable. The pancreas and gastrointestinal tract showed no significant abnormalities.

The adrenal glands had the usual shapes, although they were partially embedded within the renal capsules. They appeared relatively large (right $4.5 \times 4.5 \times 0.8$ cm.; left $6.5 \times 4.0 \times 0.6$ cm.). However, the cortices were thin and the weight of each was only about 4.5 gm. The outer cortical zone was pale yellow, suggesting depletion of lipids, while the inner zone was dark brown. The adrenal medullary tissue was not remarkable.

The kidneys together weighed 250 gm. and exhibited only moderate fetal lobulation. The pelves, ureters, and urinary bladder were essentially normal.

The vagina was 2 cm. in length. The uterus had a total length of 1.8 cm. including the cervix, which was larger than the corpus. The uterine tubes were correspondingly infantile and formed the superior ridges of the broad ligaments. At the site of the usual location of the ovary on the posterior aspect of each broad ligament was a firm yellow-gray band of tissue 2.5 cm. in length, 0.2 to 0.4 cm. in width, and less than 0.2 cm. in thickness. The medial poles of each "ovary" merged with a small cord extending to the uterus and consisting of a slight condensation of the meager fibrous tissue of the broad ligaments. No other gonadal tissue could be identified despite a careful search of the abdominal, pelvic, and inguinal regions. There were a few minute cysts at the free margin of each mesosalpinx near the fimbriae of

the uterine tubes.

Samples of ribs, vertebrae, and calvarium were moderately osteoporotic.

The brain weighed 1190 gm. The gyri, particularly in the frontal region, were small and showed increased convolutions suggesting slight microgyria rather than atrophy. The cerebral arteries were unusually thin-walled and delicate for this age, and no atherosclerosis was evident. Cut surfaces of the brain showed no gross abnormalities. The pineal body was not remarkable.

The pituitary gland weighed 445 mg. The thyroid weighed only 7.5 gm. but did not seem small in relation to the body size. Four parathyroid glands were identified in the usual locations.

Microscopic Examination

Microscopic examination revealed moderately acute degenerative changes in many of the tissues. These were in large part attributable to a terminal toxic state associated with bronchopneumonia, although difficult to distinguish from postmortem artifacts related to embalming and the postmortem interval. Sections from the heart also showed slight fibrosis of the mitral valve and epicardium, and minimal atherosclerosis of coronary arteries. The aorta was thin-walled, with a basophilic media containing many fine vacuoles. Sections from the coarctation showed the constricting ridge to consist of an exaggeration of both intima and media, with focal medial calcifications. Sections from the lungs confirmed the presence of marked bronchopneumonia. The thickened capsule of the spleen contained hyalinized fibrotic nodules. Malpighian corpuscles were less than 0.5 mm. in diameter. There was diffuse fibrosis of the spleen of slight degree. The relative number of reticulum cells in the red pulp seemed to be increased. Appropriate stains confirmed the presence of moderate fatty change of the liver and showed an increase of collagen in the portal areas consistent with minimal portal cirrhosis. The pancreas was not remarkable microscopically except for serous atrophy of adventitial adipose tissue, as noted elsewhere. The gastric mucosa was somewhat atrophic, with a greater number of lymphocytes than usual. A section of colon included a microscopic area of epithelial hyperplasia, with numerous eosinophilic leucocytes between the glands. The appendiceal lumen was obliterated by fibrous tissue. The kidneys were not especially remarkable, presenting the expected terminal and postmortem changes and slight diffuse fibrosis with sclerosis of rare glomeruli.

Not only were the adrenal glands included in the renal capsules, but in many areas kidney and adrenal were in contact without intervening fibrous tissue, and occasional dilated renal tubules were found well within the adrenal cortex. The zona glomerulosa was composed of cells with small dark nuclei and scanty eosinophilic cytoplasm. The zona fasciculata was rather narrow but had large cells with a vacuolated cytoplasm. The transition to the zona reticularis was not well defined by the pattern of the cords of cells. However, there was a rather sharp demarcation at the midregion of the cortex, due to the presence of cells in the interior half which contained abundant eosinophilic cytoplasm nearly free from vacuoles. These cells contained more brown pigment than usual in the zona reticularis at this age.

The squamous epithelium of the cervix had a thickness of only three to five small cells. The endocervix, endometrium, and myometrium were also infantile histologically.

The Fallopian tubes (i. e., Müllerian duct derivatives) had broad simple plicae, consisting of fibrous tissue covered by low columnar epithelium without definite ciliation (Fig. 1). Nearby were tortuous tubules which resembled the vestigial Wolffian bodies of women rather than the definitive masculine mesonephric derivatives (Fig. 2). Associated with these were a few bizarre microscopic structures (Figs. 3 and 4), which may also have been of mesonephric origin. In addition, there were nine cysts in the left mesosalpinx and five in the right, none greater than 3 mm. in diameter. Some of the small cysts were obviously mesonephric vestiges. The larger

Fig. 1.—Cross section of midportion of Fallopian tube; hematoxylin and eosin; × 50.

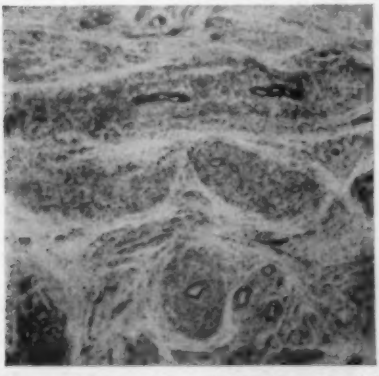

Fig. 2.—Mesonephric tubules in mesosalpinx. Hematoxylin and eosin; × 50.

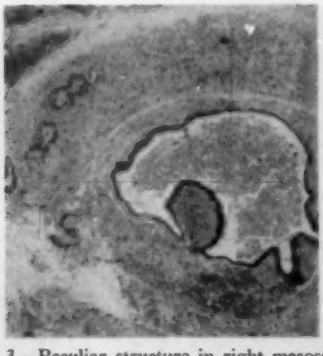

Fig. 3.—Peculiar structure in right mesosalpinx, possibly of mesonephric origin. Note the mesonephric tubules at the left. Hematoxylin and eosin; × 50.

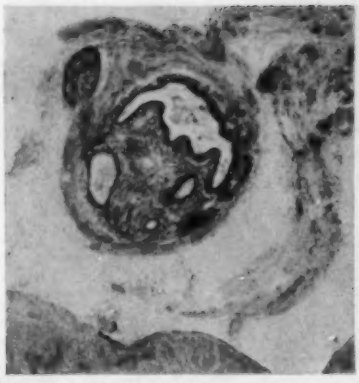

Fig. 4.—Another peculiar structure, protruding from the surface of the left mesosalpinx. Hematoxylin and eosin; × 50.

cysts had a fibrous wall and epithelial linings, which varied from a single row of squamous cells to a thick loose stratification of cells. One cyst protruding from the surface of the left mesosalpinx had a superficial resemblance to a developing ovarian follicle, since it had an eccentric mass of epithelial cells somewhat like a cumulus oophorus (Fig. 5). However, no germinal cells or derived structures could be identified in any of the sections.

The "ovarian ridges" consisted principally of rather dense fibrous tissue with a slightly whorled pattern (Fig. 6). There were no cysts or scars to suggest that oogonia had ever been present. In the deeper portions were occasional aggregations of epithelial-like cells with round or oval nuclei, a "clear" or highly vacuolated cytoplasm, and rare golden-brown cytoplasmic granules. These cells were considered characteristic of "ovarian hilus cells." Within the gonadal rudiments they were associated with a stroma which was more whorled and cellular (Fig. 7) than elsewhere. In the subjacent loose connective tissue similar "hilus cells" had much more pigment (Fig. 8). Scattered through the adnexal tissues were other aggregations of cells which could not be distinguished from these morphologically. In accord with Sternberg's descriptions,[17] these "hilus cells" were often associated with small nerves (Fig. 9), but several dis-

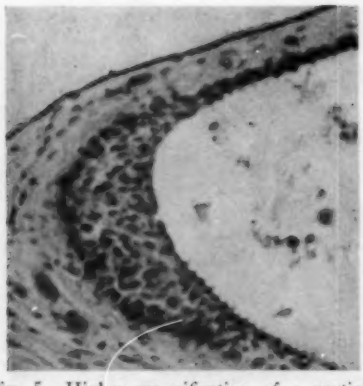

Fig. 5.—Higher magnification of a portion of one of the cysts protruding from the mesosalpinx. Note the eccentric mass of epithelial-like cells covered by a simple epithelium. Hematoxylin and eosin; × 200.

Fig. 6.—Cross section of the left "ovary." At the right upper corner is a small pedunculated irregular body attached to the serosal surface near the "ovary." Hematoxylin and eosin; × 50.

crete nodules apparently had no such relationship. There were nests of similar cells just outside the fibrous wall of the largest "parovarian" cyst (Fig. 10). The cells in all these aggregations had nuclei ranging from 5μ to 8μ in diameter, with prominent nuclear borders and scattered chromatin clumps but no definite nucleoli. None were binucleated or had cytoplasmic crystalloid bodies. Their cytoplasm was found to have an affinity for Sudan black B, probably indicating the presence of lipids which were poorly soluble in the solvents of the paraffin technique. There was marked variation in

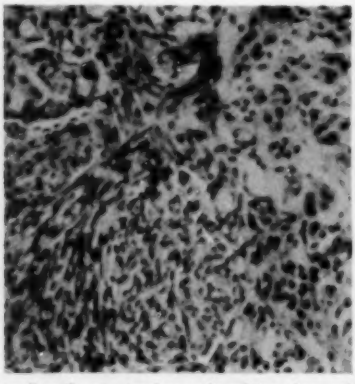

Fig. 7.—Cross section of midportion of left "ovarian ridge," showing the most cellular stroma and on the right, small groups of hilus cells. Hematoxylin and eosin; × 200.

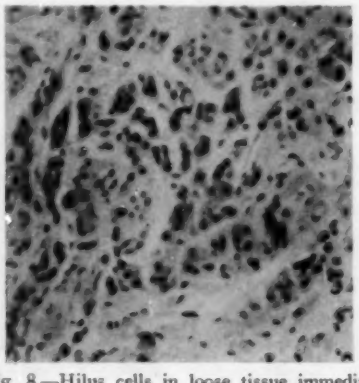

Fig. 8.—Hilus cells in loose tissue immediately subjacent to "ovarian ridge." Hematoxylin and eosin; × 200.

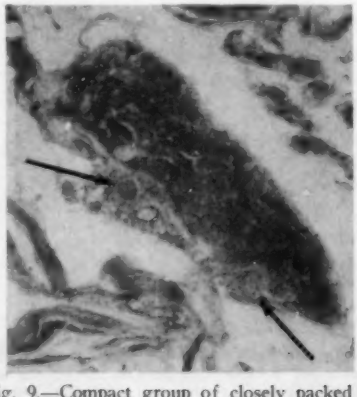

Fig. 9.—Compact group of closely packed cells found near distal end of left mesosalpinx. Arrows point to small nerves. Hematoxylin and eosin; × 50.

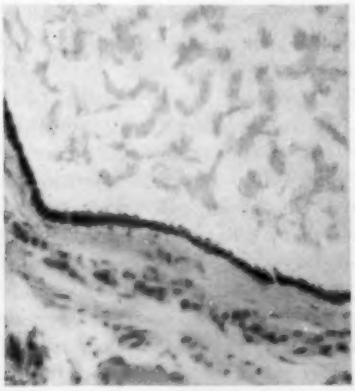

Fig. 10.—Segment of wall of largest cyst, showing nests of pigmented cells. Hematoxylin and eosin; × 200.

the quantity of the golden-brown granules in the cytoplasm. Most of the cells had little or none, although many around the cyst wall and some near nerves were packed with pigment. These granules were not doubly refractile and failed to give a positive Prussian-blue reaction for ferric iron. Giemsa stains of the formalin-fixed tissues failed to distinguish these from the granules in adrenal cortical cells, neurons, or hemosiderinophages.

In the delicate broad ligament near the lateral extremity of the left "ovary" was a yellow 1.5 mm. nodule of adrenal cortex (Fig. 11). Microscopically the three cortical zones in this nodule had the same character-

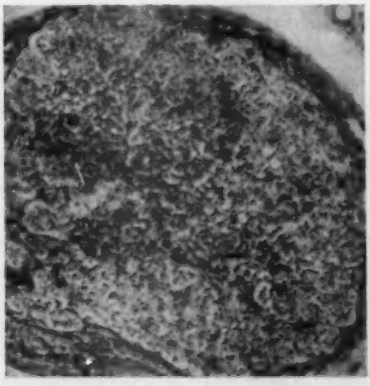

Fig. 11.—Adrenal cortical nodule, from broad ligament near fimbriated extremity of right Fallopian tube. Hematoxylin and eosin; × 50.

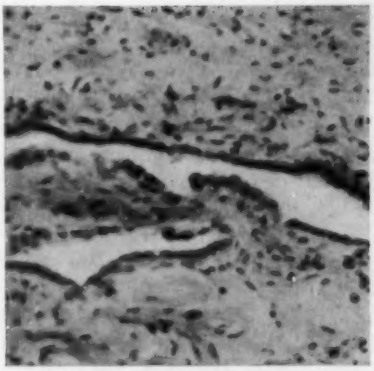

Fig. 12.—Characteristic peduncle of one of the small irregular bodies, in this case attached to a plica of the Fallopian tube. Note the abrupt transition from the columnar epithelium of the Fallopian tube to the large round cells. Hematoxylin and eosin; × 200.

istics and proportions as in the adrenal glands proper.

The largest of several collections of neurons in the nerves of the uterine adnexa formed a small ganglion consisting of a few dozen cell bodies. At least two of these neurons were binucleated. Many contained golden cytoplasmic granules.

Protruding from various surfaces of the adnexal tissues were at least eight separate pedunculated masses. Two or more of these small bodies were attached by narrow pedicles to fimbriae of each Fallopian tube. Another was found on the free border of the left mesosalpinx. One was attached to a plica in the lumen of the right Fallopian tube 10 mm. from the tip of the fimbriated extremity (Fig. 12). The largest of these irregular masses was less than 4×2×2 mm. It was smaller than the adjacent fimbriae, which it must have resembled to the naked eye. The smallest two projected from the serosal surface, one near each "ovary" (Fig. 6).

All of these peculiar bodies were covered, at least in part, by a simple epithelium (or mesothelium?), with a beaded appearance due to the rounded shapes of the constituent cells (Fig. 13). With the hematoxylin and eosin stain the abundant cytoplasm of these "superficial cells" was gray-brown and

had distinct borders. Their nuclei were rather large (5μ to 12μ), nearly round, and moderately vesicular. In some regions the stroma was apparently covered by several disorderly layers of these cells, while in places the cells were absent or detached from the surface. At least in part, this distribution was attributable to the irregularity of the surfaces and to artifacts of sectioning. However, morphologically identical cells were found well within the stroma and away from the free surfaces of these masses. No such cells were found in other tissues. These rounded "superficial cells" often had two nuclei and occasionally three or more (Fig. 14).

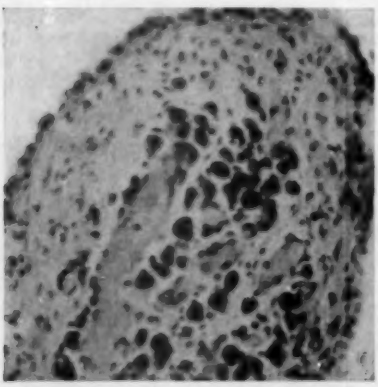

Fig. 13.—Another "irregular body," showing the large round cells over the surface. Similar cells within the stroma are not readily identified in this reproduction, but many of the presumed macrophages are prominent because of their basophilic cytoplasm. Hematoxylin and eosin; × 200.

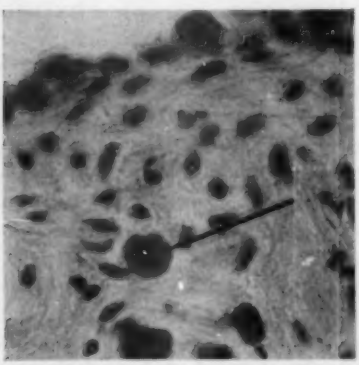

Fig. 14.—Higher magnification of upper portion of Figure 13, with "superficial cells" at the top and presumed macrophages at the bottom. The arrow points to a "superficial cell" with two nuclei. Hematoxylin and eosin; × 600.

The stroma of these irregular bodies was somewhat less fibrous and more vascular than that of the fimbriae. No nerves and no "hilus cells" were found, but in addition to the "superficial cells" these bodies contained many cells which were thought to be macrophages. Some of these presumed phagocytes were spheroidal and quite large (up to 80μ), while others were much smaller and stellate or fusiform. Their nuclei were smaller than those of the "superficial cells," and the cytoplasm was usually darker-staining and definitely granular (Fig. 14). Discrete vacuoles were prominent in many of these cells, and the cytoplasm of most had a marked affinity for Sudan black B, probably indicating the presence of considerable lipid material with low solubility in the solvents of the paraffin technique. In contrast, the homogeneous cytoplasm of the "superficial cells" stained only faintly or not at all with Sudan black B. Some of the presumed macrophages contained golden granules (hemosiderin?) which gave a strong Prussian-blue reaction.

In median and sagittal sections of the pituitary, it seemed that a slight majority of the epithelial cells of the anterior lobe were chromophobes. Basophilic cells were larger and more prominent than the eosinophilic cells, and were nearly as numerous. A few of the basophiles had large vacuoles in the cytoplasm. At the rostral aspect of the median sections was a 2 mm. nodule of small closely packed cells, most of which were chromophobes.

The thyroid had rather small follicles with slightly basophilic colloid. The parathyroid glands were not remarkable histologically, except that oxyphile cells were rather scarce. Sections from the anterior mediastinum revealed very scanty strands of thymic tissue, including scattered small spherules of epithelial-like cells (Hassall's bodies). There were also occasional minute calcifications, as well as a few cysts up to 3 mm. in diameter. Some of the larger

cysts were lined by cuboidal epithelium and contained basophilic colloid like that in the thyroid. Other cysts and small groups of epithelial cells were very similar to those in the parathyroid glands.

Sections of rib, vertebral body, and calvarium exhibited marked osteoporosis, marked serous atrophy of adipose tissue, and moderate hypoplasia of hematopoietic tissue. There were slightly increased numbers of plasma cells, but the histologic appearance was not that of multiple myeloma.

No definite abnormalities were noted in multiple sections from the brain. A section of cervical spinal cord showed more small basophilic spherules ("corpora amylacea") than usual at this age.

Sections through the left areola and nipple revealed only a few small ducts extending to the deepest layers of the dermis.

In an attempt to establish the sexual genotype, the nuclei in various tissues were studied and specimens were sent to persons with experience in this technique. They agreed that poor fixation prevented reliable counts, but some stated that the nuclear patterns of certain tissues gave the impression of the male genotype.

No peripheral blood smears were preserved, but a sternal marrow smear was available for genotypic determination by the method of Davidson and Smith.[18] This technique was modified slightly to apply to marrow smears and was found to be reliable. ‖ The nuclei of the polymorphonuclear leucocytes in the patient's marrow could not be distinguished from those of males; i.e., of 500 mature neutrophiles not one had the small appendage expected in at least 6 of 500 neutrophiles in smears from females.

Comment

The infantilism (short stature and incomplete sexual development), webbing of the neck, and cubitus valgus are characteristic of Turner's syndrome. In addition, this case presents a number of conditions which have been associated in other reports, including aortic coarctation, mental deficiency, prominent nevi, an aged facies, osteoporosis, deafness, abnormal epiphyseal fusion, and disproportionately large hands.¶ The following minor developmental anomalies apparently have not been described with this syndrome previously: bicuspid aortic valve,# elongation of the aortic arch with marked separation of the origin of the major arteries, tortuosity of the coronary arteries, deficiency of the lobar fissures of the lungs, and inclusion of the adrenal glands in the renal capsules. It is not surprising to find a bicuspid aortic valve, since this is said to be present in 75% of cases of aortic coarctation.[20] These clinically insignificant anomalies may be sequelae of mesenchymal abnormalities in early fetal life.

Certain other findings deserve comment. Although a high FSH titer is expected in a normal postmenopausal woman of 50 years, in this patient it excludes pituitary hypogonadism. Contrary to previous reports, the pituitary gland was found to have a relative increase in the number of basophilic cells. While this might be attributed to the unusually advanced age, the 61-year-old patient described by Randerath[21] was said to have had hyperplasia of small eosinophilic cells.

In the present case there was no evidence of significant functional or morphologic derangement of the thyroid. The largest parathyroid gland was slightly more than the usual size, but the total amount of glandular tissue did not seem excessive. The paucity of oxyphile cells might be considered a feature of "infantilism," since they are said to increase with age.[22] However, it is claimed that they are completely absent in the young child.

The 17-ketosteroid excretion determinations and the Kepler-Power-Robinson water

‖ Beckner, E.; Hughes, W. L., and Nelson, B. M.: Unpublished data.

¶ Reference 11, p. 4.

Schürmann's case[19] apparently had a valvular deformity secondary to rheumatic carditis.

test indicated the presence of at least some of the adrenal cortical activities. The repeated finding of eosinopenia made it impossible to evaluate the effect of administered corticotropin, but may in itself be significant. It is noteworthy that eosinophiles and their precursors had at least a normal representation in the marrow smears and sections. The physical and psychologic stresses in this case could very well have resulted in marked adrenal cortical stimulation. (No comment was made regarding rather low fasting eosinophile counts in previous case reports.*) The adrenal cortex has been considered hypoplastic in some of the relatively few autopsied cases,† although the inconstant reduction in weight is even less impressive when the size of these patients is considered. Moreover, the moderately narrow zona fasciculata of the present case might be attributed to the terminal illness. The increased pigment in the zona reticularis is in contrast to other reports and may be related to age.

The data pertaining to the illnesses of the patient's last years are not fully understood. Perhaps the anemia was due to chronic rectal bleeding, complicated by malnutrition. Dietary deficiencies might also account for intermittent diarrhea, hepatic abnormalities, transient ascites and edema, and possibly even the mental changes late in life.

The "infantile" genitalia were like those described and illustrated in previous reports,‡ with gonadal anlagen consisting of small strips of fibrous tissue. In this case, unlike a few others,[25] there was no evidence that germ cells had ever been present. The numerous small aggregations of "hilus cells" probably did not exceed the total amount of such tissue that is distributed in the larger adnexal tissues of a normal woman. As Sternberg has emphasized, these hilus cells are morphologically similar to the interstitial cells of the testis.[17] They have been found in increased numbers in some women exhibiting masculinization, and were said to be present or even hyperplastic in several cases of "ovarian agenesis with androgenic manifestations."§ However, it is not yet proved that these cells contain or produce androgens. In the present case slight facial hirsutism was the only suggestion of masculinization. The association of hilus cells and nerves is intriguing and no doubt has functional and/or embryologic significance. The relationship of these cells and the similar paraganglionic cells remains uncertain.

The finding of a small nodule of adrenal cortex in the adnexa is not considered remarkable. The binucleated and pigmented neurons in the adnexal tissues are probably of no significance, but the pigment is said to increase with age, whereas binucleated neurons are said to be common in the child.[26]

The irregular bodies attached to various adnexal surfaces are puzzling. The "superficial cells" of these small bodies might represent some sort of transition between the serosal mesothelium and the epithelium of the Müllerian ducts. Another speculation is that they are vestiges of the so-called "germinal epithelium," for some reason sequestered from the gonads.

It is unfortunate that genotypic studies were handicapped by poor preservation of the nuclei. Since previous studies of "ovarian agenesis" have shown that either genotypic pattern may be present, inconclusive results in this case would seem unimportant. However, the theory that the condition results from chromosomal defects in the zygote suggests the possibility that some of these patients might prove to have nuclear patterns differing from either sex, or perhaps intermediate. The observation ‖ that "quite typical female chromatin" was present in certain nuclei but not in most others from this patient may have significance, although the genotypic studies in

* References 23 and 24.

† References 4, 9, 19, 21, and 25.

‡ References 4, 7, 9, and 11.

§ References 11 and 13.

‖ Barr, M. L.: Personal communication to the author.

this case must be regarded as inconclusive by this method, and the bone marrow smear is entirely compatible with the male genotype. Moreover, several dozen genotypic determinations have already been published,[13] and no inconclusive or intermediate chromosomal patterns were noted.

Nevertheless, it is believed that genotypic determinations are of considerable interest in patients with possible chromosomal defects involving sexual development. The peripheral blood smear and the oral mucosa smear[27] are convenient methods for this purpose clinically.

Because patients with Turner's syndrome often have nuclear patterns which resemble those of males, the term "ovarian agenesis" is inappropriate. "Gonadal agenesis" has also been used, but to many physicians this would connote complete absence of the gonads.[28] On the other hand, "dysgenesis"¶ applies to faulty development, although it may refer to abnormalities which are not necessarily hypoplastic. The phrase "gonadal infantilism" would also seem to be fitting, especially since the condition has to be distinguished from the well-known "pituitary infantilism." However, it might be objected that these patients are not truly infantile, and that in fact premature aging is often observed. Certainly the gonadal vestiges are not merely "infantile."

"Ateliosis" is an old and almost forgotten synonym of "infantilism" with a more literal connotation of incomplete development. Thus the phrase "gonadal ateliosis" could be applied to the characteristic abnormalities of the gonads, as well as to the associated clinical syndrome of "infantilism." But whatever descriptive titles are used for the condition, it is likely that the common variety with webbing of the neck and cubitus valgus will continue to be known as "Turner's syndrome," at least parenthetically, until the etiology is established.

¶ References 11 and 13.

Summary

A 50-year-old woman had characteristic features of Turner's syndrome, including small stature, sexual immaturity, webbing of the neck, cubitus valgus, and stigmata of lesser degree. Clinical studies confirmed the expected high excretion of FSH, moderately low 17-ketosteroid excretion, and other findings of primary hypogonadism. This mentally deficient patient was well adjusted to a child-like status until late in life, when she had a marked change in behavior, perhaps in relation to physical illness of uncertain etiology. Death was attributed to bronchopneumonia.

Autopsy disclosed minor anomalies not previously reported with this condition, but the principal abnormalities were classical. The vagina, uterus, and Fallopian tubes were rather small and the "ovaries" rudimentary. Minute aggregations of "hilus cells" were found in the adnexal tissues. Projecting from various adnexal surfaces were a number of small bodies covered by epithelial (mesothelial?) cells. These irregular bodies apparently have not been described previously.

Studies of the nuclei in the embalmed tissues were not satisfactory, but an antemortem bone marrow smear indicated a genotype like that of males, as often found in cases of "ovarian agenesis."

Dr. R. Palmer Howard and Dr. H. H. Turner, of the University of Oklahoma School of Medicine, assisted in the endocrinologic studies of the patient and offered helpful suggestions for the preparation of this paper. Dr. Ray Northrip provided the bone marrow smears. Drs. M. L. Barr, B. Lennox, E. Marburger, and W. O. Nelson expended considerable effort to establish the genotype.

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Electron Microscopic Studies of a Hamster-Adapted Virus

Isolation from a Patient with a Common Cold

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Introduction

The common cold (acute coryza, acute rhinitis) is attributable to the infection of the upper respiratory tract with a virus. The symptoms and signs indicate the presence of an acute, but transitory, alteration in the physiology of the mucous membrane of the upper part of the respiratory tract, particularly that lining the nose and the paranasal sinuses (Horsfall [1]). All of the etiologic factors are not yet completely understood, but there is good evidence indicating that one or more viruses initiate a cold.

Kruse [2] and Foster [3] successfully transmitted colds experimentally to man by means of filtered materials. More than 10 reports have confirmed their findings, while 5 others have failed to confirm them. Dochez and associates [4] first reported the successful transmission of the common cold to chimpanzees by means of filtrates. Dochez and associates [5] reported the cultivation of the agent in tissue culture medium, and Kneeland and co-workers [6] described the cultivation of the agent on the chorioallantoic membrane of the chick embryo. Pollard and Caplovitz,[7] Topping and Atlas,[8] and Ward and Proctor [9] described cultivation of the virus in the allantoic sac of embryonating chicken eggs. Andrews and colleagues* have been unsuccessful in attempts to cultivate their strains in the chick embryo. All mammalian species tested, except man and chimpanzees, appeared to be insusceptible to infection with the etiologic agent [10] until Reagan and associates † showed that the suckling hamster was susceptible to several common cold virus strains. Colds experimentally induced by the intranasal inoculation of bacteriologically sterile filtrates or chick embryo passage material closely simulate natural colds and show all the features of the naturally acquired disease.

In a previous study (Reagan and associates [14]), by electron microscopy of filtered infected nasal and throat washings from an adult affected with a common cold, spherical virus-like particles with a diameter of 80μ-90μ were observed. The virus was capable of inducing a cold in one chimpanzee. These virus-like bodies were not observed in normal nose and throat washings.

Materials and Methods

Nasal and throat saline washings were obtained during the first two days of a cold from a worker in our laboratory. The clinical picture was as follows: slight sneezing in the early morning, which was followed by copious watery nasal discharge, and also watering eyes during the day. In the late afternoon he experienced light headache and chilly sensations. The following day he felt malaise, with cessation of the nasal discharge during that afternoon. During the course of the cold, he never had cough, purulent nasal discharge, or occluded nostrils.

Two lactating hamsters and their sucklings were placed in individual metal boxes with bedding. The sucklings were 6 days old. The lactating hamsters from Group 1 were given 0.03 ml. intranasally of

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* References 10-11.

† References 12-13.

filtered nose and throat washings from the affected patient. The sucklings from Group 2 were likewise given filtered nose and throat washings from a normal patient. (This patient had had no sign of a common cold for the past year.) Dog Checkers were fed to each of the two groups daily, along with a large bottle of water for drinking. Blood was taken, 24 hours after exposure, by the intracardiac route from sucklings of Group 1, pooled, and added to 2 ml. of heparinized isotonic saline. Blood from the control suckling Group 2 was treated likewise. The cell suspension (treated 10 seconds in purified water U. S. P. to remove some of the hemoglobin in the cell) from each group was then employed in electron microscope studies. The same procedures were carried out on erythrocyte suspensions taken from suckling hamsters of the infected Group 1 and control Group 2 at time intervals of 48, 72, 96, 120, 144, and 168 hours. From the fourth to the fifth day, suckling hamsters from Group 1 showed cold virus symptoms, such as running nose and wheezing, and the nostril area was swollen, boggy, and inflamed. (Four of the sucklings showed these symptoms, and the other two remained normal throughout the experiment.) The sucklings from the control Group 2 remained normal. All hamsters were discarded after a 14-day period.

Each specimen was prepared for electron microscope examination by placing small drops of the erythrocyte-heparinized saline-purified water suspension on Parlodion (collodion) film supports, which had been prepared two weeks previously. The films were dried and shadowed with gold (Williams and Wyckoff [16]) at arc tangent 4/12 and examined under the RCA electron microscope, Type EMU.

Upon examination by electron microscopy of the erythrocytes taken at the designated time intervals from control Group 2, no virus-like particles could be seen. However, upon examination of the erythrocyte suspension taken from Group 1, definite virus-like particles could be seen. However, the surface of the erythrocytes obtained at the time interval of 72 hours. These virus-like particles are shown in Figures 2 and 3. The virus-like particles had the same shape and size as those demonstrated by electron microscopy of the egg-adapted cold virus strain (Reagan and associates [13]). Figure 1 shows a representative erythrocyte from the 72-hour control-group bleeding with no virus-like particles present.

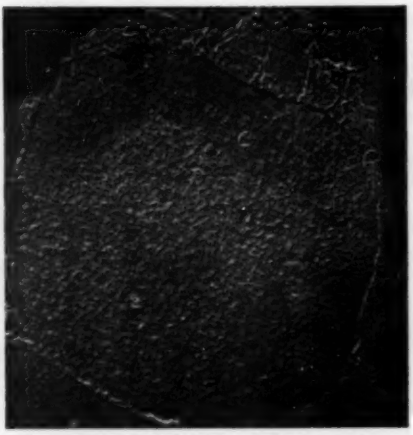

Fig. 1.—Electron micrographs of erythrocytes from suckling hamsters exposed nasally to normal nose and throat washings showing no virus-like particles; 72-hour bleeding; films shadowed with gold; arc tangent 4/12; ×30,000.

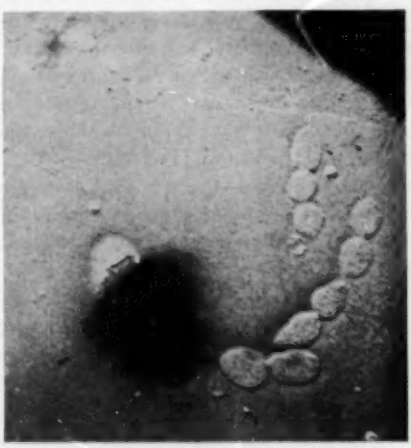

Fig. 2.—Electron micrographs of erythrocytes from suckling hamsters infected nasally with Chang cold virus; 72-hour bleeding; film shadowed with gold; arc tangent 4/12; × 40,000.

Summary

Electron microscope studies of erythrocytes from suckling hamsters exposed intranasally to the Chang strain of the common cold virus showed the virus to be present in, on, and around the erythrocytes only from the 72-hour-interval bleeding. No virus-like particles could be demonstrated from the other interval bleedings or from the control group bleedings. Four

Fig. 3.—Electron micrographs of erythrocytes cytes from suckling hamsters infected nasally with Chang cold virus; 72-hour bleeding; film shadowed with gold; arc tangent 4/12; × 60,000.

out of six of the suckling hamsters from the exposed group showed cold virus symptoms from the fourth to the fifth day, such as running nose, wheezing, and swollen, boggy, and slightly inflamed nasal area. The other two suckling hamsters and the sucklings from the control group appeared normal and showed no common cold symptoms.

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Experimental Pulmonary Hypertension and Arteriosclerosis

Absence of Intimal Reaction in Pulmonary Arteries of Rabbits with Right Ventricular Hypertrophy Following Pulmonary Vascular Obstruction by Nonthrombotic Material (Plastic Beads)

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An important recent advance in the investigation of arteriosclerosis has been the production of arteriosclerotic lesions in the small pulmonary arteries of rabbits by the repeated intravenous injection of blood clots.* The full significance of these experiments has not yet been determined, and further study is necessary before definitive conclusions can be drawn. One problem that requires exploration is the pathogenesis of the arteriosclerotic lesions that can be produced in pulmonary arteries by thromboembolism.

Fibrotic pulmonary arterial lesions produced in the small pulmonary arteries of rabbits by the repeated intravenous injection of blood clots can be divided on the basis of their appearance into two general types. One type is a clearly recognizable organized thrombus characterized by a loose meshwork of fibrous tissue through which pass multiple vascular channels. The other type is characterized by concentric or eccentric fibrous intimal thickening, characteristic of pulmonary arteriosclerosis. The pathogenesis of the first type of lesion is quite clear. It results simply from the ingrowth of fibrous tissue into a thrombus. The pathogenesis of the second type is not clear. These arteriosclerotic lesions may represent the end-stage of another form of organization of thrombi or may be the result of a reaction of the vessel wall to hemodynamic alterations (such as hypertension) produced by the artificially induced vascular obstruction.† If the latter possibility is the correct one, it seems likely that similar lesions could also be produced by types of obstructing bodies other than blood clot.

The purpose of this report is to present a description of the pulmonary arteries in rabbits that have received repeated intravenous injections of nonthrombotic obstructive bodies (plastic beads).

Materials and Methods

Fifty-five young adult New Zealand white rabbits of both sexes were used as experimental animals. They were housed in an air-conditioned animal room and fed 100 gm. Purina rabbit pellets daily and given water ad libitum.

The plastic beads‡ used for intravenous injections are spherical, smooth-surfaced, and vary in size from 50μ to 150μ. It is estimated that each gram of these beads consists of a million individual particles. They are a Lucite polymer and have proved to be insoluble in body fluids, but readily soluble in xylene used in the preparation of tissue sections. The beads were suspended (20 mg/cu. ml.) in a 5% dextrose solution and were injected repeatedly into ear veins of designated rabbits (bead group) through an 18-gauge needle. Total quantities given to each rabbit are listed in Table I. Some died as a result of the procedure, and others were killed with intravenous formalin.

In the early stages of the experiment the individual doses of beads varied from 5 to 500 mg. However, it soon became apparent that almost all

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* References 1-6.

† References 2 and 7.

‡ The plastic beads were supplied by the courtesy of the Polychemicals Department of E. I. DuPont deNemours & Co., Washington, W. Va.

adult rabbits could tolerate 200 mg. but that more than 200 mg. was occasionally lethal. Hence, in the latter part of the experiment each dose was 200 mg. The intervals between injections also varied considerably in the early part of the experiment, but in the latter part an attempt was made to inject all rabbits three times each week. However, it became increasingly difficult to introduce a large-bore needle into the traumatized veins, so that the intervals between injections were still variable.

Comparable material was obtained from rabbits that had not been subjected to any experimental procedures (noninjected, control, group). Additional comparison was made with material and data obtained from rabbits used in a previous experiment[4] that had been given repeated intravenous injections of blood clot (clot group §).

§ This group was referred to as the "water-clot group" in the previous report. These 15 rabbits were given six weekly injections of intravenous blood clot, and at the same time 25 ml. of water was introduced into their stomachs through a gastric tube. The maximum time elapsed between the first injection and death of the rabbits used in this experiment was seven weeks.

Autopsies were performed on all the rabbits, and selected portions of tissue, including the entire thoracic contents, were fixed in 10% formalin for at least five days. Each heart was then separated from the lungs and pericardium by sectioning the great veins at the pericardium and cutting across the aorta and pulmonary artery at the superior borders of their valve cusps. The atria were removed by cutting along the atrioventricular groove. The right ventricle was then separated from the interventricular septum. The weight of the right ventricle without the interventricular septum divided by the weight of the left ventricle including the interventricular septum will be referred to hereinafter as the "right ventricular-left ventricular ratio."

One block of pulmonic tissue was taken from each upper and each lower lobe for paraffin sections. Sections from all paraffin blocks were stained with aldehyde fuchsin-Van Gieson-iron hematoxylin,[8] and some sections were also stained with hematoxylin and eosin. Frozen sections were cut on selected tissue and stained with oil red O.

In order to determine how effectively the plastic beads obstructed the flow of blood, one additional experimental procedure was used. Ear chambers

TABLE 1.—*Data from Rabbits Given Intravenous Injections of Plastic Beads*

Rabbit No.	Total Beads, Mg.	No. of Injections	Days Since 1st Injection*	Heart Wt., Mg.	R. V.† Wt., Mg.	L. V.† Wt., Mg.	R. V./L. V.‡
1	3525	26	350	8,200	1800	5200	35%
2	3350	17	300	11,425	2800	6900	41%
3	3200	17	320	7,400	2000	4350	46%
4	2675	13	350	7,250	1950	4000	49%
5	2500	12	244	7,010	1520	4430	34%
6	2110	9	105	10,550	2300	6500	35%
7	2100	10	218	6,700	1600	4100	39%
8	2050	17	250	5,800	1100	4000	28%
9	2000	10	270	7,850	1650	5150	32%
10	1820	17	220	7,750	1500	4700	32%
11	1800	13	107	6,950	1440	4240	34%
12	1630	17	150	6,460	1510	3530	42%
13	1080	13	82	5,450	1100	3500	31%
14	1000	17	150	8,000	1400	5150	27%
15	1500	16	126	6,190	1330	3850	35%
16	1500	6	97	8,700	2300	5800	40%
17	1250	6	169	6,550	1480	4300	34%
18	1150	5	219	5,800	1350	3600	38%
19	1000	3	60	6,300	1050	4200	25%
20	1000	5	57	5,900	1400	3550	39%
Average	1972	12	192	7,312	1627	4602	35.8%

* Days between first injection of beads and death.
† R. V. is right ventricle and L. V. is left ventricle.
‡ The method for deriving the right ventricular-left ventricular ratio is given in the text.

TABLE 2.—*Right Ventricular-Left Ventricular Ratio of Noninjected (Control) Group*

Rabbit No.	R. V./L. V.	Rabbit No.	R. V./L. V.	Rabbit No.	R. V./L. V.	Rabbit No.	R. V./L. V.
21	27%	29	27%	37	29%	45	27%
22	28%	30	28%	38	32%	46	29%
23	30%	31	28%	39	32%	47	29%
24	23%	32	29%	40	29%	48	25%
25	28%	33	28%	41	30%	49	30%
26	26%	34	29%	42	28%	Ave.	28.4%
27	31%	35	29%	43	29%		
28	26%	36	30%	44	29%		

TABLE 3.—*Average Right Ventricular-Left Ventricular Ratios in Each of Three Groups of Rabbits*

	%
Untreated (control) group	28.4
Water-clot group	31.6
Bead group	35.8

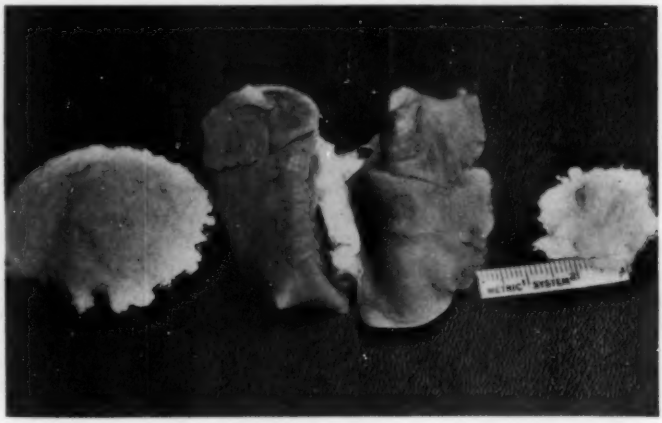

Fig. 1.—Gross photograph of the lungs of a rabbit between two piles of plastic beads. The piles of beads represent the maximum and minimum total amounts received by the rabbits in the experiment. Reduced to ¾ actual size.

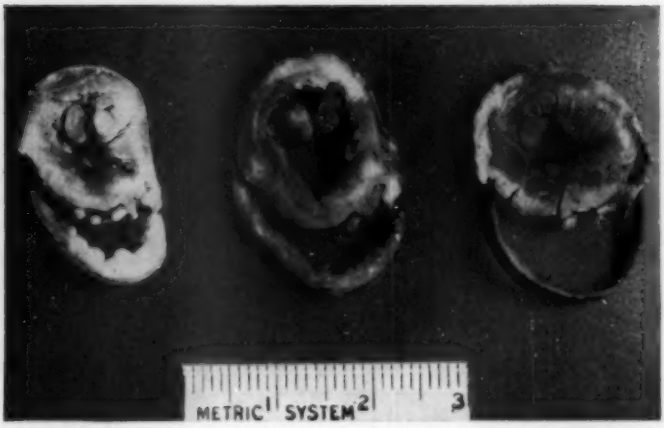

Fig. 2.—Sections of the midportions of the ventricles of three rabbits. This photograph shows the grossly evident right ventricular hypertrophy present in (from the left) Rabbits 12 and 16, as contrasted with the heart of an uninjected (control) rabbit (on the right). Reduced to ¾ of mag. ×2.

were inserted into rabbits' ears, using the technique of the Clarks,[9] and beads were injected directly into the central artery of the rabbit's ear.‖

Results

Data are presented in detail in the Tables and illustrated in the Figures 1-4 about here.

‖ The rabbits' ear chambers were prepared by Mr. Erwin Rabin, a senior medical student at Washington University.

Gross Observations

The average of the right ventricular-left ventricular ratios was 35.8% in rabbits that had received beads and only 28.4% in the noninjected controls. The difference between these two figures is highly significant ($p<0.01$). The corresponding ratio for a group of rabbits (used in a previous experiment[4]) that had received repeated intravenous injections of blood clot was 31.6%. This figure is significantly greater ($p<0.01$) than that in the control group and significantly less than that in the bead group ($p=0.02$). No other significant gross abnormalities were noted.

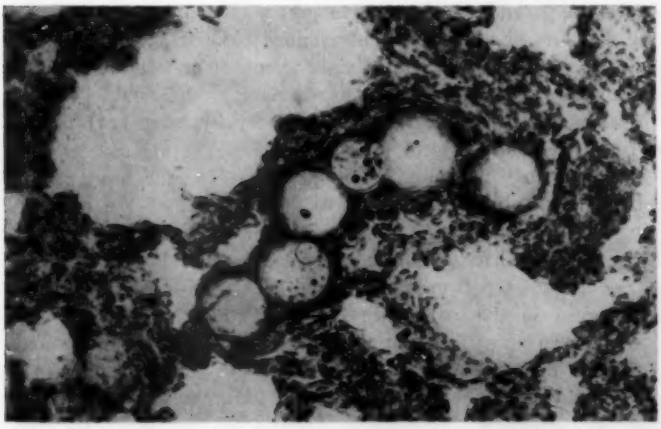

Fig. 3.—Photomicrograph of a frozen section of the lung from Rabbit 12. The plastic beads are arranged in single file in a pulmonary arteriole. Hematoxylin-eosin stain; reduced to 73% of mag. × 220.

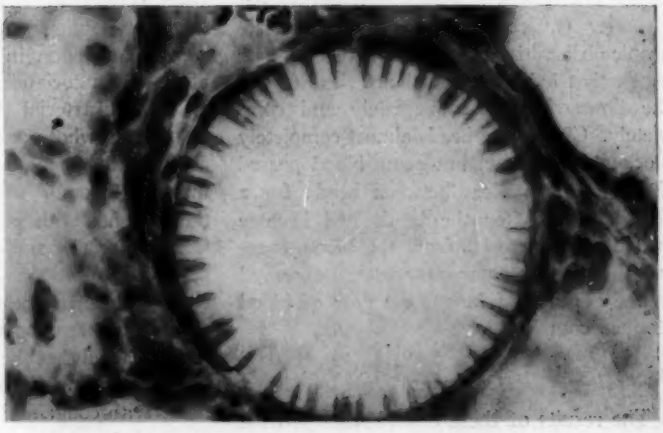

Fig. 4.—Higher magnification of a single plastic sphere in a pulmonary vessel illustrating the absence of significant reaction in the vessel wall. The vascular lumen continues to the upper left and contains a few erythrocytes. Hematoxylin-eosin stain; reduced to 73% of mag. × 920.

Microscopic Observations on Pulmonary Vessels

A slight degree of fibrous and fibroelastic intimal thickening of the small pulmonary arteries and arterioles was common in both the control and the bead group. These changes were most frequently seen at the site of branching of vessels. The extent and severity of this fibrous intimal thickening were similar in the control group and the bead group. We did not encounter advanced intimal lesions, although such lesions are frequent in the pulmonary arteries of rabbits that have received repeated intravenous injections of blood clot.[4]

The beads were readily identified in frozen sections. They were not seen in paraffin sections, since they are soluble in xylene, which was used in the preparation of the tissue. However, the site at which a bead had lodged was identifiable because a perfectly round clear space remained. Beads were found singly and in clusters. Some clusters consisted of as many as 8 or 10 beads. In most instances the beads completely filled the lumen of the vessel. None was found outside vessel walls. No inflammatory reaction was noted. The beads rapidly became coated with a thin film of proteinaceous material, which filled the spaces between beads. After the beads had been present for a long time, the material surrounding the beads had the appearance and staining characteristics of collagen, although only occasional fibroblasts were seen.

At this late stage the beads were apparently rather firmly attached to the wall and occasionally appeared to be retracted to one side. This thin coating of the beads represented the only tissue reaction that was noted. Thrombosis did not occur either distally or proximally. Often the lumen was larger at the site occupied by the bead than at adjacent levels, suggesting that the bead had entered the vessel with such force that it had stretched the wall.

From the histologic appearance in the tissue sections, it appeared that in most instances the beads had effectively occluded the lumens of their containing vessels. This impression was confirmed by injecting beads into arteries passing through an ear chamber (making possible in vivo microscopic observations) in a rabbit's ear. Single beads that lodged in small arteries stopped the flow of blood immediately and completely. Clusters of beads almost completely blocked blood flow, but some blood passed through the crevices between beads for a few hours. After the beads had become coated with material from the blood (presumably the proteinaceous material observed in the sections of lung), the flow of blood stopped completely.

Comment

The results of these experiments provide strong support for the concept that the arteriosclerotic lesions produced in rabbits by the repeated injection of blood clot ¶ are actually organized thrombi. If the arteriosclerotic lesions (in rabbits subjected to embolization with blood clot) result simply from pulmonary hypertension or other hemodynamic alterations associated with obstructing bodies, such lesions should have been present in rabbits subjected to embolization with plastic beads, but no such lesions were found. In most of the rabbits receiving beads, the quantity of obstructing bodies, as judged by the number observed in the sections, exceeded considerably the number of obstructing lesions found in a comparative group of rabbits that had received intravenous blood clot. Comparison of the right ventricular-left ventricular ratios (an objective, rough guide to the degree of pulmonary hypertension) in the bead group with those in the clot group [4] provides confirmation for the bead group. Furthermore, the duration of the obstruction was far greater in most of the rabbits in the bead group than in those in the clot group (average 192 days for the bead group and only 45 days for the clot group [4]).

The results of these experiments are consistent with the results obtained by us in a recent study of arteriosclerosis of the small pulmonary arteries in autopsied patients with congenital heart disease.[5] Arteriosclerotic lesions of the pulmonary arteries were as common in patients whose anomalies are associated with low or normal pulmonary arterial pressures as they were in patients whose anomalies are often associated with pulmonary hypertension. Pulmonary thrombi were common in all of the patients with congenital heart disease. On the basis of these observations plus the presence of lesions that could represent transition forms between clearly recognizable thrombi and arteriosclerotic plaques, we concluded that the arteriosclerotic lesions in the small pulmonary arteries of the patients with congenital heart disease probably resulted from the presence of the thrombi and not from hypertension.

Summary

In recent experiments arteriosclerotic lesions have been produced in the small pulmonary arteries of rabbits by the repeated intravenous injections of blood clots. The question arises as to whether these arteriosclerotic lesions result directly from the presence of the thrombi or indirectly from pulmonary hypertension or other hemodynamic alterations associated with the thrombotic obstructing bodies. In the experiments reported in this paper we have attempted to answer this question by subjecting rabbits to repeated intravenous injec-

¶ References 1-6.

tions of nonthrombotic obstructive bodies (plastic beads).

The number of obstructive bodies in the pulmonary arteries, the degree of right ventricular hypertrophy (an objective, rough guide to the degree of pulmonary hypertension), and the duration of the obstruction were much greater in the rabbits receiving beads in this experiment than the corresponding values in most other experiments in which pulmonary arteriosclerosis had been produced by the intravenous injection of blood clots. In spite of the advanced degree of pulmonary arterial obstruction, marked right ventricular hypertrophy, and the long duration of the obstruction, no pulmonary arteriosclerotic lesions were produced in rabbits that received only intravenous injections of plastic beads.

Hence, the conclusion is reached that the arteriosclerotic lesions produced by thromboembolism in rabbits are not simply the result of pulmonary hypertension or other hemodynamic alterations associated with obstruction. It seems likely that these arteriosclerotic lesions are actually organized thrombi.

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The Acute Effects of Beta$_3$ Thienylalanine in the Adult Male Albino Rat

Observations on Nitrogen Balance, Antibody Formation, and Tumor Growth

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Many competitive inhibitors of specific amino acids have been synthesized and their influence upon bacterial growth has been extensively studied,[1] but with the exception of ethionine * and β_2-thienylalanine,[4] few studies in mammals have been reported. Recently the acute and reversible effects of β_3-thienylalanine, a specific inhibitor for phenylalanine, upon both the growth of young rats and the protein repletion of protein-deficient adult rats have been summarized.† No studies of this phenylalanine antagonist in the adult well-nourished animal have been recorded.

The dietary amino acid requirements for growth [7] and optimum weight gain of protein-depleted rats [8] are quantitatively different from the essential amino acid pattern which is necessary to maintain body weight and nitrogen balance.[9] Some of this difference between the amino acid requirements for growth, or tissue repletion, and the requirements for maintenance is probably due to the different quantities of the various amino acids needed for synthesis of large quantities of muscle, liver, and blood proteins as compared with the requirements for maintaining the *status quo*. A part of this difference may also be due to the availability of stores of amino acid in the protein reserves of the adult well-nournished animal.‡ Thus, in the normal animal an effective amino acid inhibitor would not only block the utilization of dietary amino acids but would also interfere with the utilization of essential amino acids liberated during the normal break-down of tissue proteins.

The present study is concerned with the acute effects of a phenylalanine antagonist in the normal adult rat. The experiments were planned so that the influence of β_3-thienylalanine upon body weight, nitrogen balance, the synthesis of specific antibody globulin, and neoplastic growth could be observed within a few days after the addition of the substance to the diet. In order to insure control of other dietary ingredients fed with the inhibitor, a maintenance amino acid ration was used and the rations were given by stomach tube.

Materials and Methods

Plan of Experiment.—Six experiments were performed, all with similar dietary regimens. The first three experiments were identical in plan and similar in content except for certain dietary control groups. They were designed to demonstrate whether or not the effects of β_3-thienylalanine on nitrogen balance and antibody formation were consistently reproducible from experiment to experiment. Well-nourished animals of the same age and

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Throughout this study a DL-mixture was used which was supplied by Prof. Karl Dittmer, Department of Chemistry, Florida State University, Tallahassee, Fla.

* References 2 and 3.

† References 5 and 6.

‡ References 10 and 11.

of comparable weights were prepared for each experiment by giving increasing amounts of a liquid ration in which the amino acids were supplied by a protein hydrolysate. After this preliminary period of eight days, the animals were divided into comparable groups, each of which was fed a different ration designed to test the effects of β_3-thienylalanine in varying amounts as compared to several control nutritional states simultaneously. When antibody formation was to be measured, all of the animals were given a single intravenous injection of antigen after the diets had been fed for two days. In the first three experiments, they were started on a four-day period of nitrogen balance determinations after two days. The experimental rations were fed over a period of eight days except in the fourth experiment, and the animals were bled for antibody determinations on the fourth and sixth days following the antigen injections. The animals were then killed, and tissues were taken for histologic study. In the fourth experiment, the animals were bled for antibody titers and were killed on the sixth experimental day (the fourth day after antigen injection) in order to provide histological sections at the time when the spleen shows a maximal histological reaction to the antigen.[12] The intravenous injection of antigen, as well as the nitrogen balance determinations, was omitted in the experiments designed to study the effects of β_3-thienylalanine on tumor growth (Experiments 5 and 6). In these two experiments the animals were injected with a tumor suspension during the preliminary tube-feeding period, and the tumors were measured daily during the experimental period. In all the experiments the animals were weighed daily.

Animals.—Adult male albino rats of the Sprague-Dawley strain were used in all six experiments. The rats for each particular experiment were of the same age and of comparable weights, though the average weights in the six experiments varied from 300 to 550 gm.

Synthesis of Inhibitor.—β_3Thienyl-DL-alanine was synthesized by the method reported by Dittmer[13] as modified by Shapira, Shapira, and Dittmer.[14] After two recrystallizations the product was identical with previously prepared samples in regard to microbiological activity and elementary analysis. It was found to be free of other amino acids as determined by paper chromatography.

Diets and Feeding.—In order to insure control over the amino acid composition of the experimental rations a mixture of pure amino acids present at maintenance level (MAA) was used (Table 1). The level at which the MAA mixture was included in the diets was calculated according to the average weight of the rats in each experiment, 3.07 gm. of MAA/kg/rat/day being necessary to produce nitrogen balance and maintain body weight.[§]

The MAA diets used in all six experiments were essentially the same, with minor differences in the amounts of dextrin, MAA mixture, and corn oil (Table 1). The corn oil was varied to meet the caloric requirements of the average rat in the experiment. In order to allow the rats a calorie intake of 1600 cal/M^2/day, the surface area was determined using the formula $M^2 = 12.54 (\text{weight})^{0.4}$.§ The protein-free ration differed from the MAA diet in that dextrin replaced an equal weight of the amino acid mixture. The dry diet was blended with enough water to make a homogeneous liquid mixture of a consistency suitable for tube-feeding the required daily calories

§ References 15 and 16.

TABLE 1.—*Composition of Basic Diet and of Maintenance Amino Acid Mixture Used for Tube-Feeding Experiments in Adult Previously Well-Nourished Rats*

Tube-Fed Maintenance Ration*	
Ingredient	Gm./100 Ml.
Dextrin	20.63
Corn oil	5.63
MAA	3.43
Cellulose	2.63
Salt mix	2.11
Vitamin mix	0.53
50% Choline	0.32
Percomorph liver oil	0.16
Water	67.7

Water-Soluble Vitamins	
Ingredient	Mg./100 Ml.
Nicotinic acid	2.12
Ca pantothenate	1.06
Pyridoxine HCl	0.53
Riboflavin	1.06
Thiamine HCl	0.53

Maintenance Amino Acid (MAA) Mixture	
Amino Acid	Mg./Kg./Rat/Day
DL - Alanine	298.7
L - Arginine HCl	265.7
DL - Aspartic acid	324.7
L - Cystine	18.3
L - Glutamic acid	1474.1
Aminoacetic acid	26.0
L - Histidine HCl, HOH	38.3
DL - Isoleucine	330.0
L - Leucine	103.3
L - Lysine HCl, HOH	80.0
DL - Methionine	93.3
DL - Phenylalanine	76.7
DL - Threonine	136.7
DL - Tryptophan	28.3
L - Tyrosine†	76.8
DL - Valine	256.7
Total	3607.7

* Composition for Experiment 3. Basic composition varied slightly in each experiment, depending on the weight of the rats being used.

† In the first experiment 329.3 mg. of tyrosine and 1221.7 mg. of glutamic acid were used. The tyrosine was reduced to the tabulated value in all subsequent experiments to decrease the possibility of dietary tyrosine reducing the effectiveness of the β_3-thienylalanine. Glutamic acid was increased by a comparable amount.

TABLE 2.—*Average Rat Weights and the Dietary Variables in Each Experiment*

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
No. rats used	35	35	25	12	10	21
Av. rat wt., gm	350	380	550	450	300	300
MAA/rat/day,gm.	1.14	1.39	1.98	1.98	1.98	1.06
Cal./rat/day	50.6	73.7	89.5	89.	89.5	60.8
1 × phenylalanine*/rat/day gm.	0.025	0.030	0.042	0.042	0.042	0.025

* Since the molecular weights of phenylalanine and β_3-thienylalanine are similar, the quantities of the inhibitor (1 × to 4 ×) incorporated in each experimental ration represent multiples of the phenylalanine in that ration.

TABLE 3.—*Average Body Weight, Nitrogen Balance, and Serum Protein Data, with Standard Errors**
Experiments 1, 2, and 3

Diet Group	8-Day Wt. Change, Gm.			4-Day N-Balance, Mg.			8-Day Serum Protein, Gm, /100 Cc.†
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1
20% Hydrolysate	+5(±1)	+10(±3)	.....	+144(±41)	+676(±90)	.....	6.9 (±0.05)
MAA	− 8(±2)	− 5(±2)	+ 7(±1)	0 (±16)	0 (±6)	0 (±21)	6.5 (±0.1)
MAA + 1X β_3TA	−16(±2)	−12(±2)	.....	− 65(±15)	− 43(±36)	.....	6.2 (±0.09)
MAA + 1X β_3TA + 4XPA	−12(±2)	.....	.....	− 85(±6)	.....	.....	6.4 (±0.04)
MAA + 1X β_3TA + 6XPA	.....	−18(±5)	.....	.....	− 67(±31)	.....	.....
MAA + 3X β_3TA	.....	.....	−12(±4)	.....	.....	−411(±33)	.....
MAA + 4X β_3TA	−41(±1)	.....	.....	−594(±10)	.....	.....	5.1 (±0.2)
MAA + 3X β_3TA + 6XPA	.....	.....	−21(±3)	.....	.....	−476(±55)	.....
MAA + 6XPA	.....	.....	+12(±4)	.....	.....	−1 (±17)	.....
MAA without PA	.....	−15(±3)	.....	.....	−49 (±24)	.....	.....
Protein-free basal	−21(±2)	−12(±1)	.....	−148(±15)	−234(±11)	.....	6.1 (±0.1)
Fasted	−81(±4)	−82(±3)	.....	−491(±57)	−431(±14)	.....	6.1 (±0.1)

* These data are the averages of five rats in each diet group. Each group of five rats was comparable to the other groups used in that experiment in age, weight, and previous dietary history. All groups within any given experiment were tested simultaneously, and no animals were used for more than one experiment.
† Serum protein concentration determined on blood obtained at killing, eight days after the diets were started.

and amino acid allotments. The amounts of β_3-thienylalanine and phenylalanine added to the MAA diets were varied within as well as between experiments (Tables 2 and 3). When phenylalanine was added in excess, it was added as a multiple of the daily amount normally found in the MAA mixture and it replaced an equal weight of dextrin. The β_3-thienylalanine varied from 25 mg/rat/day to 100 mg/rat/day.

Vacuum-concentrated protein hydrolysate (Aminosol ‖) was used during the preliminary tube-feeding period because of its solubility and adequate assortment of essential and nonessential amino acids. The amount was comparable to the concentration of MAA used subsequently. In some of the experiments this hydrolysate was also incorporated at a 20% level in a liquid ration designed to serve as a control stock diet.

The animals were fed the experimental rations by stomach tube two times a day, as nearly 12 hours apart as practical. The amount of diet given per feeding varied from 15 ml. in the first and fifth experiments to 20 ml. in the other four.

Nitrogen Balance.—The animals were kept in individual metabolism cages for the four-day nitrogen balance determinations. The urine and feces were collected daily from each animal, and the daily collections were pooled for the four-day period. The cages were washed carefully with purified water at the end of the four days, and the washings were added to the pooled urine samples. The combined urine collections and washings were diluted volumetrically. The four-day fecal collections were dried, weighed, and thoroughly pulverized with a mortar and pestle. Two 1 ml. samples of each day's diet were diluted volumetrically. Kjeldahl nitrogen determinations in triplicate were made on aliquots from the urine, feces, and diets, and from these the four-day nitrogen intake, total four-day nitrogen excretion, and the nitrogen balances were calculated.

Antigen Injection and Antibody Determinations.—Antibody determinations were performed during

‖ Donated by Abbott Laboratories, North Chicago, Ill.

the first four experiments by use of the hemolysin method described by Cannon and co-workers.[17] An amount of 1 ml. of a 0.25% suspension of washed sheep erythrocytes in 0.86% saline was injected in the tail vein of each rat. An amount of 1 ml. of blood was collected from each rat on the fourth and sixth days following the injection. The bloods were allowed to clot, and the sera were separated and kept frozen until the hemolysin titers were measured. Bloods which had been obtained for base-line titers prior to the start of the experiment were similarly treated. In the fourth experiment, 1 ml. of a typhoid vaccine was injected intravenously at the same time as the sheep erythrocytes. In this experiment four-day agglutinin titers were determined on the sera as well as the hemolysins, and no six-day titers were performed, since the animals were killed after the four-day bleedings.

Histology.—Tissues were taken at the time of killing for histological study in the last three experiments. Samples of liver, spleen, thymus, mesenteric lymph node, bone marrow, kidney, and adrenal were fixed in 10% buffered formalin and in Carnoy's fixative. The formalin-fixed sections were stained with hematoxylin and eosin. Popper's modification¶ of the Unna-Pappenheim methyl-green-pyronine stain was utilized to stain the Carnoy-fixed tissues. Samples of the spleen, lymph node, testis, liver, and bone marrow from the fourth experiment were fixed only in Carnoy's and stained with methyl-green-pyronine. The tissues taken in the tumor experiments were the same as those listed above, with the addition of sections of tumor and small intestine.

Tumor Injection and Measurement.—In the last two experiments, the animals were injected on the second day of the preliminary period with 0.2 ml. of a 1:2 sterile saline homogenate of Jensen sarcoma; the injection was made into the anterior thigh muscles. The tumor tissue was removed aseptically from rats which had been similarly injected with the sarcoma one week before. Only grossly bloodless areas of tumor tissue were used to prepare the inoculum. The tumors were measured daily with calipers, with an attempt to obtain measurements in three perpendicular dimensions, and the size was expressed in cubic centimeters.

Results

The results of the four-day ntrogen balance determinations, weight changes, and the serum determinations performed in the first three experiments are summarized in Table 3. Here it can be seen that even the lower levels of β_3-thienylalanine resulted in a weight loss and a negative nitrogen balance, which was not reversed by the addition of more phenylalanine. The higher level of β_3-thienylalanine caused a more significant weight loss and a greater negative nitrogen balance, comparable to that of the fasted animals. The higher level also led to a marked acute hypoproteinemia. Addition of a greater amount of phenylalanine in the second experiment did not reverse the effect of the β_3-thienylalanine. The omission of phenylalanine in the second experiment resulted in weight loss and negative nitrogen balance comparable to that seen in the animals given 30 mg. of β_3-thienylalanine in this experiment. The weight loss seen on the MAA diets in the first two experiments is probably due to a slight caloric deficiency.

The variability of the nitrogen balance data reflected in the large standard errors observed in some of the groups probably results from varying maintenance amino acid requirements of individual rats. In spite of the standard preliminary diet period, some of the animals apparently had greater phenylalanine requirements than others. Under these circumstances one would expect a variable effect of β_3-thienylalanine upon nitrogen balance from animal to animal.

The hemolysin titers are graphically shown in Figures 1, 2, and 3. Antibody formation was not affected acutely by the protein-free ration or fasting as compared with the 20% hydrolysate diet. Although the MAA ration seemed to depress the antibody output moderately in the first experiment (Fig. 1), there was no evidence of this in the other two experiments (Figs. 2 and 3). The addition of β_3-thienylalanine consistently depressed the four- and six-day hemolysin level in all three experiments, with the larger amounts having the greater effects. Additional phenylalanine added to the ration containing the β_3-thienylalanine abolished this effect. The reversal was observed whether the additional phenylalanine

¶ Popper, H.: Personal communication to the authors.

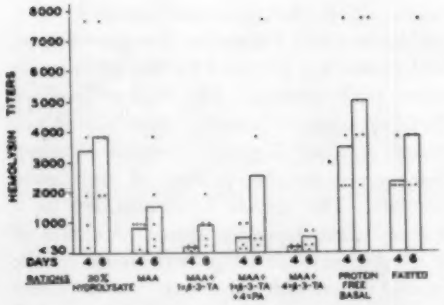

Fig. 1 (Experiment 1).—Anti-sheep-erythrocyte-hemolysins expressed as the reciprocal of the highest dilution of serum showing complete hemolysis. The bars represent mean values at four and six days after antigen injection, and the individual titers are indicated by points.

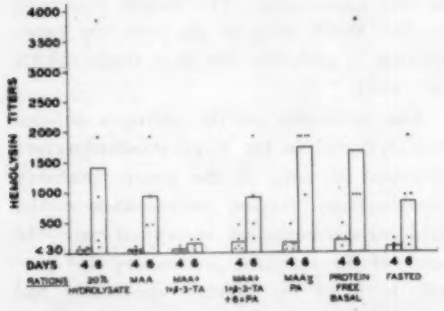

Fig. 2 (Experiment 2).—Anti-sheep-erythrocyte hemolysins under various conditions of acute dietary imbalance. Results recorded as in Figure 1, except that the ordinate scale is different.

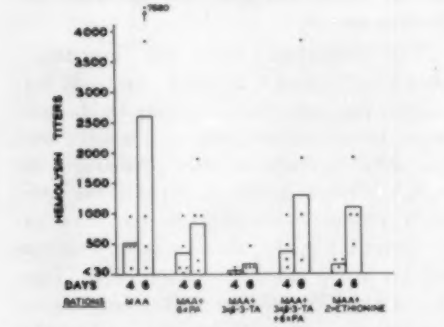

Fig. 3 (Experiment 3).—Anti-sheep-erythrocyte hemolysins four and six days after injection of antigen. Results expressed as explained in Figure 1.

was given in four or six times its usual amount.

Neither the removal of phenylalanine (Fig. 2) nor the addition of extra phenylalanine to the MAA ration (Fig. 3) had any definite effect on humoral antibody concentration in the absence of the inhibitor. Although there was a large variation in antibody concentration from animal to animal, particularly in the six-day titers of the control rats, the effects of similar rations in the three experiments were so consistent that there can be little doubt of the acute suppression of antibody formation by the β_3-thienylalanine.

TABLE 4.—*Comparison of the Average Four-Day Agglutinin Levels with the Degree of Proliferation of Pyroninophilic Cells in the Red Pulp of the Spleen*
Experiment 4

Diet Group	4-Day Typhoid Agglutinins	4-Day Spleen Red Pulp Proliferation
MAA	320	3+
MAA + 3× β_3-TA	70	1+
MAA + 3× β_3-TA + 6×PA	480	3+
Chow	560	4+

The fourth experiment was performed primarily to obtain tissues for histological study. The animals were killed four days after the antigen injection. Antibody formation to both sheep erythrocytes and typhoid vaccine was greatly inhibited by the addition of β_3-thienylalanine in this experiment, and this fact was correlated with a marked depression of pyroninophilic cell response in the red pulp of the spleen, a response which is most conspicuous at four days.[12] This cellular effect of β_3-thienylalanine was also largely reversed by increasing the phenylalanine in the ration containing the inhibitor (Table 4). The illustrations (Figs. 4, 5, and 6) show the typical response of the spleen at four days in the animals given MAA, MAA plus 4× β_3-thienylalanine, and MAA plus 4× β_3-thienylalanine plus 6× phenylalanine. In contrast to this change seen in the red pulp of the spleen, β_3-thienylalanine produced no appreciable effect on the histology of the bone marrow, lymph nodes, testis, gastrointestinal tract, or the Malpighian corpuscles of the spleen.

The fifth and sixth experiments were performed to find out if β_3-thienylalanine had

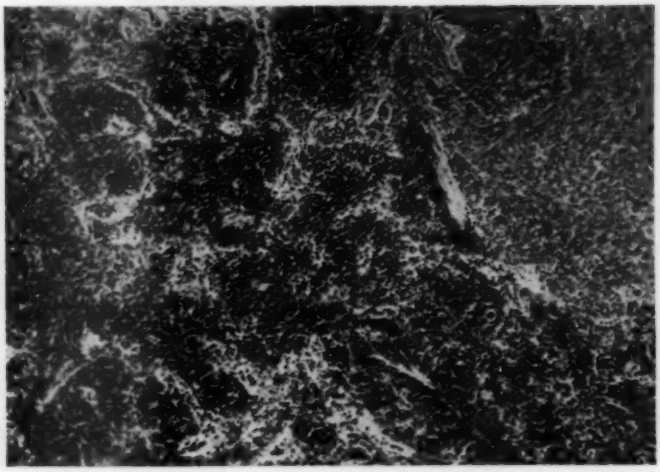

Fig. 4.—The appearance of the red pulp of the spleen four days after a single intravenous antigen injection in a rat receiving the MAA ration. Note the numerous clumps of dark-staining cells which show a brilliant red cytoplasm with the methyl-green-pyronine stain. These nonphagocytic cells appear to be derived from reticular cells. They actively proliferate by mitosis between the second and fourth day following antigen injection, and then they rapidly disappear. Most of them bear little resemblance to mature plasma cells, and few of them appear to mature into plasma cells. Reduced about ⅓ from mag. × 165.

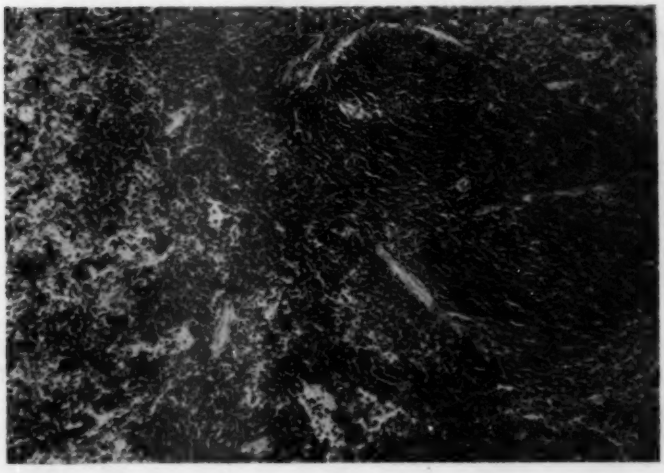

Fig. 5.—The red pulp of the spleen shows only a little proliferation of pyroninophilic cells in this rat, which received 100 mg. of β_2-thienylalanine per day added to the MAA ration, starting two days before antigen injection. This is the characteristic appearance of the spleen of this group four days after antigen injection. Note the apparent lack of damage to the Malpighian corpuscle. Reduced about ⅓ from mag. × 165.

any selective effect on the growth of Jensen sarcoma. Histologically, the inhibitor-containing ration produced definite depression in numbers of mitotic figures in the tumors. Furthermore, the cells of the inhibitor-treated tumors in Experiment 5 had smaller and sometimes less distinct nucleoli and more prominent chromatin (Figs. 7 and 8), and the treated tumors showed a higher percentage of necrotic tissue. The sixth experiment confirmed these findings and, in addition, showed that this effect appeared to be reversible when additional phenylalanine was added to the diet. The comparative tumor growth in rats receiving the MAA ration and those fed the MAA

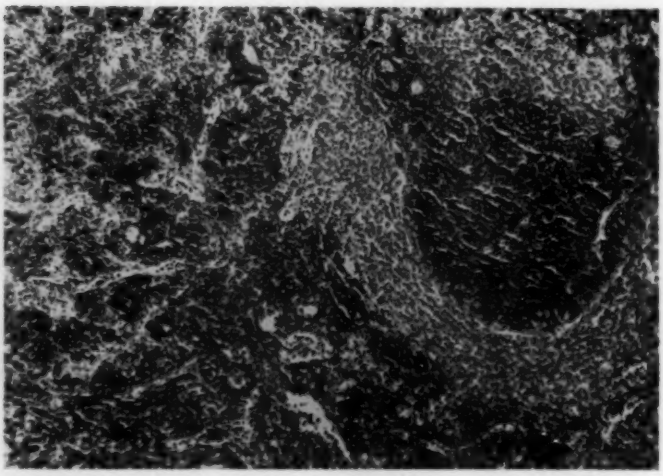

Fig. 6.—This animal is from the group which received 100 mg. of β_3-thienylalanine per day but which also received six times the usual daily quantity of phenylalanine. Four days after antigen injection the spleen shows almost the same pyroninophilic cellular hyperplasia as seen in the animals fed the MAA ration. Reduced about ⅓ from mag. × 165.

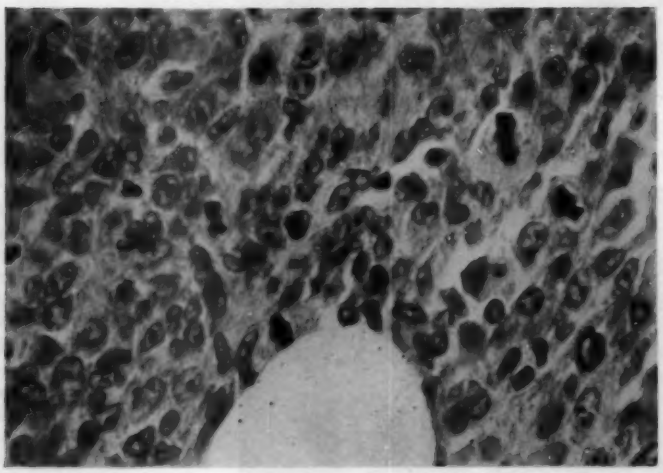

Fig. 7.—The histological appearance of the Jensen sarcoma from a rat killed eight days after feeding of the MAA ration was begun. Note the numerous mitoses, the large chromatin-poor nuclei, and the prominent nucleoli. Hematoxylin and eosin; reduced about ⅓ from mag. × 1135.

plus β_3-thienylalanine diet in Experiment 5 is shown graphically in Figure 9. The mitotic counts determined by counting the figures in 12 representative high-power fields of the microscopic sections of the tumors from seven inhibitor-fed and six MAA-fed rats in Experiment 6 are as follows: The rats receiving the β_3-thienylalanine showed 7 mitoses per four high-power fields, while the tumors from the MAA-fed rats showed 20 mitotic figures per four high-power fields. The results from each of three separate sets of counts showed excellent agreement. Additional experiments have confirmed these preliminary observations with this and other transplantable tumors, and these results will be published in detail later.

Comment

The acute effects of incorporating β_3-thienylalanine into a balanced amino acid ration under conditions of constant dietary intake by adult previously well-nourished rats may give some indication of the mode of action of amino acid inhibitors. When β_3-thienylalanine was added to a ration in four times the usual concentration of

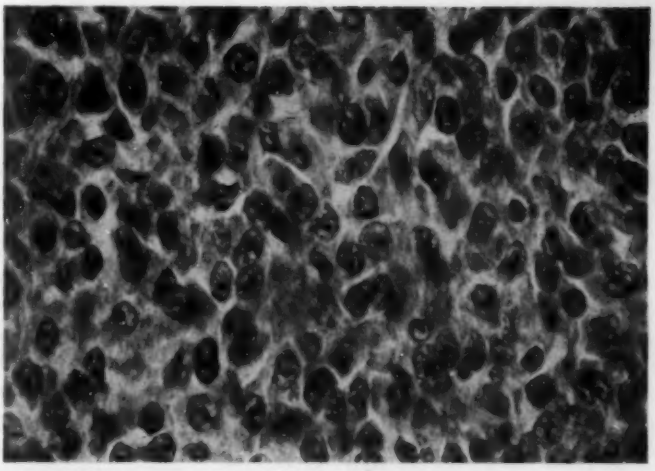

Fig. 8.—A section of a Jensen sarcoma from a rat which received 80 mg. of β_3-thienylalanine per day added to the MAA ration for eight days. Note the almost complete absence of mitotic figures, the increase in chromatin in the nuclei, and the less prominent nucleoli. Hematoxylin and eosin; reduced about ⅓ from mag. × 1135.

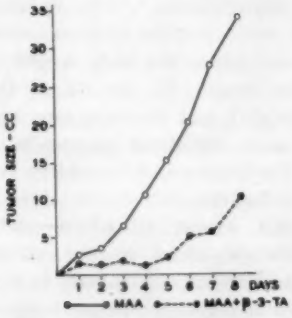

Fig. 9 (Experiment 5).—The mean volumes of Jensen sarcomas in two groups of five rats each receiving identical amino acid rations except for the presence or absence of β_3-thienylalanine in the diet. The eight-day volumes are calculated from measurements of the tumor after removal at autopsy. The antemortem values are calculated from three-dimensional measurements in situ.

phenylalanine, the rats lost more weight and showed a severer negative nitrogen balance than did rats fed a protein-free ration. This suggests that, under these circumstances, this amino acid antagonist not only inhibits the utilization of dietary phenylalanine but also interferes with the reutilization of phenylalanine, from the "protein-reserves."

In previous studies it has been shown that neither net protein synthesis [18] nor maintenance of nitrogen balance [19] can occur if one essential amino acid is absent from the diet, even for a short time.[20] Therefore, one might expect a similar blocking of the utilization of amino acids from the metabolic pool in the presence of an effective competitive amino acid inhibitor. This expectation is strengthened by the fact that rats fed a larger amount of the phenylalanine inhibitor developed a severe acute hypoporoteinemia during an eight-day period, whereas fasting or the feeding of a protein-free ration for the same length of time had little effect on the serum protein concentration. It seems likely, therefore, that this type of study may offer a new method for interrupting protein metabolism swiftly, in a way which would take several weeks with a single amino acid deficiency or a low-protein ration. There was little evidence of acute toxicity from this regimen, either in terms of histological damage to normally proliferating cells (spermatogonia, gastrointestinal epithelium, or hemopoietic cells) or in terms of total animal morbidity or mortality. Therefore, this may be a promising way to study the acute effects of intrinsic amino acid imbalance in certain clinical and experimental problems related to nephritis, hypertension, tumor metabolism, etc.

The acute depression of antibody formation by this phenylalanine inhibitor may be viewed similarly. Ordinarily a low-protein ration has to be continued several weeks

before there is any great decrease in antibody fabrication in response to a single antigen injection. As emphasized by Cannon [21] this is presumably due to the fact that the relatively small quantities of amino acids necessary for γ-globulin synthesis can be furnished in balanced form from the break-down of labile liver and muscle protein for a considerable period after inadequate amino acids are present in the diet. This phenomenon has been demonstrated experimentally.[22] Failure to recognize this principle has been responsible for confusing conclusions of other investigators.# They have suggested that since brief or mild protein deficiency has no appreciable effect on antibody levels there is therefore no relationship between protein depletion and antibody formation.

The results of the present study may be contrasted with those obtained with short-term dietary protein lack, either resulting from a single amino acid deficiency or total dietary nitrogen lack or fasting. Administration of an amino acid antagonist may, under certain circumstances, acutely interrupt the synthetic reactions necessary for antibody formation. That this process requires cellular proliferation is becoming more apparent.* Hence, it is not surprising that the major histological characterisic of β_3-thienylalanine administration in the antigen-injected rat should be depression of the characteristic proliferation of pyroninophilic cells in the red pulp of the spleen.[12] In the rat this organ is apparently responsible for most of the antibody formation in response to a single intravenous injection of particulate antigen.[28]

Recent experiments in this laboratory have indicated that the chronically protein-depleted rat whose antibody-forming capacity is impaired also shows a marked decrease in splenic cellular proliferation in response to intravenous antigen.[29] Similarly, the acute depression in antibody formation seen after total-body irradiation may be correlated with a depression in cellular proliferation in the spleen.† Why β_3-thienylalanine can depress this cellular reaction following antigenic stimulation without appreciable effect on other rapidly proliferating normal cells is not clear.

A puzzling aspect of these experiments was the failure of additional dietary phenylalanine to reverse the negative nitrogen balance and the tendency toward weight loss observed when β_3-thienylalanine is added to the maintenance amino acid ration, particularly since the effect on antibody formation was completely reversed by addition of more phenylalanine. Furthermore, additional phenylalanine has been shown to support weight gain in the protein-deficient rat fed β_3-thienylalanine.[6] This failure in the present study may be more apparent than real, inasmuch as the body weight changes were not great (2% to 3% of the total body weight) and in obese rats might not reflect acute variations in protein equilibrium. Furthermore, it is evident from the nitrogen balance data of the third experiment that almost all of the additional phenylalanine added to the maintenance amino acid ration was excreted in the urine. The greater negative nitrogen balance noted when six times the maintenance quantity of phenylalanine was added to the inhibitor-containing ration in the second experiment as compared with the group receiving four times the usual quantity of phenylalanine in the first experiment gives added support to this view. Therefore, one would not expect a large increment in nitrogen balance to result from reversal of the β_3-thienylalanine effect by means of additional dietary phenylalanine, even though some additional essential amino acids were spared for protein synthesis. This would be particularly true if the inhibitory effect of β_3-thienylalanine on protein synthesis were somewhat selective in its action. The histological observations suggest that some tissues were relatively unaffected by the phenylalanine inhibitor. Although the microsope is a

References 23 and 24.

* References 12 and 25-27.

† References 12 and 30.

rather crude tool for evaluating the state of protein metabolism, it is evident that spermatogonia, the gastrointestinal mucosa, and the hemopoietic tissues were still proliferating after eight days of dietary phenylalanine inhibitor, even when the inhibitor was given in large quantities. This suggests that these tissues may be able to utilize phenylalanine in a combined form or may possess intracellular protein stores or anabolic mechanisms such as transamination which will enable metabolism, including mitosis, to continue for some time after the inhibitor is added to the diet. On the other hand, the mitotic acivity and protein (antibody) synthesis of the cells which proliferate in response to an external stimulus, such as anigen injection, apparently are suppressed by the presence of β_2-thienylalanine in the diet.

A similar mechanism was presumably demonstrated when β_2-thienylalanine suppressed transplantable tumor growth. Although there was little evidence of damage to normal tissues or of generalized toxicity, the neoplastic cells showed a marked decrease in mitosis, increased necrosis and certain characteristic changes in the cytoplasm and nucleus of the remaining viable cells. This seems to represent a selective action on the proliferative and metabolic activity of the neoplastic cell.

Jacquez and co-workers have studied a number of amino acid antagonists and have summarized their effects on the growth and morphology of normal and neoplastic mouse cells in tissue cultures.‡ Although β_2-thienylalanine and β_3-thienylalanine both showed evidence of competitive inhibition of phenylalanine, there was evidence of only slight differential toxicity of these substances on tumor tissue as compared with their effects on heart tissue. The toxicity of these substances for the heart tissue could largely be prevented by addition of phenylpyruvate, whereas this was not true for sarcoma 241, apparently because the heart cells could transaminate the phenylpyruvate to furnish additional phenylalanine while the tumor could not.

Studies in vivo in the mouse showed that sarcoma T241 growth could be markedly inhibited by the subcutaneous injection of β_2-thienylalanine and that substantial numbers of giant tumor cells occurred in the neoplasm.[33] Although a similar reduction of neoplastic growth could be obtained by fasting the mice, it is important to note that the histological characteristics of the neoplasms were not similarly altered. When phenylpyruvate was injected along with the inhibitor, Jacquez and co-workers were unable to prevent weight loss in the mice receiving the β_2-thienylalanine while producing depression of neoplastic growth. However, their results suggest that β_2-thienylalanine produced selective damage to the neoplasm. This selective action, as judged by changes in body weight as compared to tumor weight, might have been more apparent if the caloric intake of the mice could have been maintained by stomach tube-feeding. The mice studied by Jacquez showed atrophy and histological suppression of the hemopoietic and generative organs, in contrast to the results reported in the present study in rats receiving adequate calories with the inhibitor. This suggests that their treated animals may not have consumed their stock ration while receiving the inhibitor. Furthermore, as suggested by Jacquez and co-workers,[33] control of the quantity of phenylalanine in the ration, as was possible with the tube-fed MAA ration, may aid in demonstrating the selective action of β_2 or β_3-thienylalanine on neoplastic tissue. In fact, in recent experiments we have been able to show retardation of neoplastic growth by incorporating small amounts of β_3-thienylalanine in amino acid rations from which phenylalanine was omitted.§ A study of the mechanism of the selective action of β_3-thienylalanine on tumor growth and its possible synergistic effect when combined

‡ References 31 and 32.

§ Bristow, E. C., and Wissler, R. W.: Unpublished observations.

with other compounds which have promise in cancer chemotherapy is in progress.

Summary

The addition of variable quantities of β_3-thienylalanine to a tube-fed maintenance amino acid ration during a seven- or eight-day period caused a variable acute depression in body weight and nitrogen balance of the previously well-nourished rat. The weight loss and negative nitrogen balance were increased in proportion to the quantity of inhibitor in the ration. In contrast to results in protein-depleted rats, these effects were not reversed by additional phenylalalnine in the rations.

The feeding of the inhibitor also was characterized by an acute depression in antibody formation following a single intravenous injection of antigen given two days after the inhibitor was incorporated in the diet. This effect of β_3-thienylalanine was effectively reversed by addition of more phenylalanine to the amino acid ration, and it could not be duplicated by a similar period of fasting or by feeding rations free of amino acid nitrogen or phenylalanine. Histological studies of spleens of rats treated with β_3-thienylalanine during the antibody-forming period indicated a depression of the pyroninophilic cellular proliferation in the red pulp of the rat's spleen, a proliferation which is usually associated with antibody formation to a particulate intravenously injected antigen. There was little evidence of damage to any normally rapidly dividing rat cells.

When β_3-thienylalanine was fed to rats with well-established Jensen sarcoma transplants, there was marked retardation of tumor growth accompanied by reduction in mitosis, nuclear changes, and an increase in necrotic tumor. This effect could be prevented by incorporating more phenylalanine in the diet.

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Effect of Polysaccharides on the Formation of Granulation Tissue

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In view of the effect produced by high-polymer levan and dextran in inhibiting acute inflammation,* it was considered important to investigate the effect of these polysaccharides on the processes of repair and on the formation of granulation tissue (chronic inflammation).

The effect of these compounds on the phenomena of chronic inflammation was considered interesting also because of the similarity between the effect on acute inflammation of these polysaccharides and that of cortisone. The present report deals with the effect of high-polymer polysaccharides and partially degraded polysaccharides of the type used for plasma-volume expansion on the phenomena of chronic (proliferative) inflammation as exemplified by wound healing and by the peritoneal reaction to talcum.

Materials and Methods

In a preliminary experiment adult rabbits weighing about 2 kg. each were used. In all other experiments the animals were young albino mice, 16-20 gm. in weight. In the wound-healing experiments in mice, the rostral part of the back of the animal was depilated by BaS, and a transverse cut about 1 cm. in length was made with sharp scissors between the shoulder blades. The cut was made by lifting the skin and avoiding injury to the muscle fascia. The cut was approximated and closed with a Michel clip. The incision was done without anesthesia with clean instruments, which were kept in alcohol 70%, but no attempt at asepsis was made otherwise.

In the talcum experiments, a suspension of talcum in isotonic saline solution containing 5 mg. per milliliter was sterilized by autoclaving, shaken, and injected intraperitoneally into mice in the dose of 1 ml. Throughout all experiments the mice were given daily injections of 500 units of penicillin subcutaneously.

In the animals given polysaccharide, the injections were given either twice or once daily, at fixed times, intraperitoneally. The dosage varied between 20 and 60 mg. per mouse per day. The injections of polysaccharide were started on the day before the skin incision or the talcum injection. The polysaccharides were administered as 3% solutions in saline.

In most experiments native (high-polymer) levan prepared from Aerobacter levanicum[4] was used. Its molecular weight is probably greater than 10^7. Before injection the solution was heated for 15 minutes to 80 C and centrifuged at 6000 rpm for 20 minutes, and the supernatant only was used.

For comparison, in one experiment the following polysaccharide preparations were also used: (*a*) clinical levan, a degraded levan preparation[4] of an average molecular weight of about 80,000; (*b*) native dextran, produced by the Commercial Solvents Corporation (Terre Haute, Ind.), Lot No. 378, of an average molecular weight greater than 10^7; (*c*) clinical dextran (produced by the same firm), Lot D253, of an average molecular weight about 70,000.

Between three and nine days after the incision (or injection of talcum) the animals were killed with ether. In the wound-healing experiments, the skin around the incision was excised and its outer and inner surfaces were carefully examined. It was then extended on filter paper and fixed in a 10% formalin solution. The piece of tissue was trimmed after a few hours so as to produce sections perpendicular to the surgical incision.

In the talcum-injection experiments the abdomens of the killed mice were opened and carefully

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* References 2 and 3.

examined. Subsequently, adhesions or grains of talcum which were found embedded within the tissues were taken for histological examination.

The tissues were embedded in paraffin, and sections 6μ-8μ in thickness were prepared and stained by hematoxylin and eosin and Van Gieson's method; in some experiments sections were also stained by the PAS procedure, Weigert's stain for fibrin, Rinehart and Abul Haj's modification of Hale's method, and Laidlaw's reticulum method, and with 1% toluidine blue for the so-called true metachromasia.

Experiments

After a preliminary experiment, done on four rabbits, had indicated that a dose of 100 mg. of native levan daily (rabbits weighing approximately 2 kg.) retarded the growth of granulation tissue into skin incisions, the following experiments were performed:

Experiment 1.—The skin of mice was incised as described above. The levan-group mice were given twice daily a dose of 10 mg. of native levan intraperitoneally. The "control-group" mice were not given polysaccharides. Five mice of each of the groups were killed three days after the incision; the other mice (8 levanized and 11 control mice) were killed four days later (seven days after the incision).

Experiment 2.—The skin of mice was incised as described above. The mice were divided into three groups, each containing six animals. The Group A mice were given an intraperitoneal dose of 30 mg. of native levan twice daily; the Group B mice were given 60 mg. of levan once daily; Group C mice did not get any levan. Two animals of each group were killed four days after the incision; the other animals were killed three days later.

Experiment 3.—Five groups of mice (10 mice per group) had their skin incised, as described above. All the mice receiving polysaccharides were given two daily intraperitoneal injections, each containing 15 mg. of the polysaccharide. Group A mice were given clinical dextran. Group B mice were given native dextran; Group C mice received clinical levan; Group D mice, native levan. Group E mice were not given any polysaccharides. All the animals were killed six days after the incision. There were 9 animals in Group A, 7 in Group B, 10 in Group C, 7 in Group D, and 9 in Group E at the end of the experiment.

Experiment 4.—Mice were divided into three groups. Group A mice were injected intraperitoneally with talcum at the beginning of the experiment and with 30 mg. of native levan daily. Group B were given the talcum but no levan. Group C received the levan only. The animals (10 per group originally, and 9, 5, and 6 in the respective groups at the end of the experiment) were killed nine days after the talcum injections.

Experiment 5.—This was similar to Experiment 4 except that the levanized mice received 10 mg. of the polysaccharide twice daily; the animals (8 at the beginning of the experiment; 6, 8, and 8 in the respective groups at the end) were killed seven days after the talcum injection.

Experimental Results

Wound-Healing Experiments.—Native levan and native dextran caused a partial inhibition of the formation of granulation tissue. The inhibition was noticeable both macro- and microscopically already three and four days after the incision, but could best be studied at six to nine days (Figs. 1 and 2). The effect was most marked in the mice receiving 60 mg. of levan per day, less so in those receiving 30 mg., and least in those receiving 20 mg. a day. The effect was more marked in animals receiving the levan every 12 hours than in those which received the same dose in a single daily injection. It should be noted, though, that, on the one hand, even with the 20 mg. per day the effect was obvious, while, on the other hand, not even the highest dosage inhibited completely the growth of granulation tissue.

Microscopically, in most animals treated with the high-polymer saccharides, part of the original exudate which filled the gap formed by the incision was not yet organized, even in nine days. The granulation tissue in these mice had a distinctive appearance. It was very loose, rich in intercellular material, and relatively poor in cells. In many areas the granulation tissue was made up mainly of large, foamy histiocytes. These histiocytes often appeared similar to the cells of leproma, with multiple tiny vacuoles. In other places the vacuoles seemed to have coalesced, and the cells had large, empty-looking cytoplasm with irregular outline (Figs. 3 and 4). The foamy change in the histiocytes involved not only the area of the incision but also the histiocytes of the skin farther away. The number of fibroblasts in this tissue was small in comparison with that in the nonlevanized animals, and

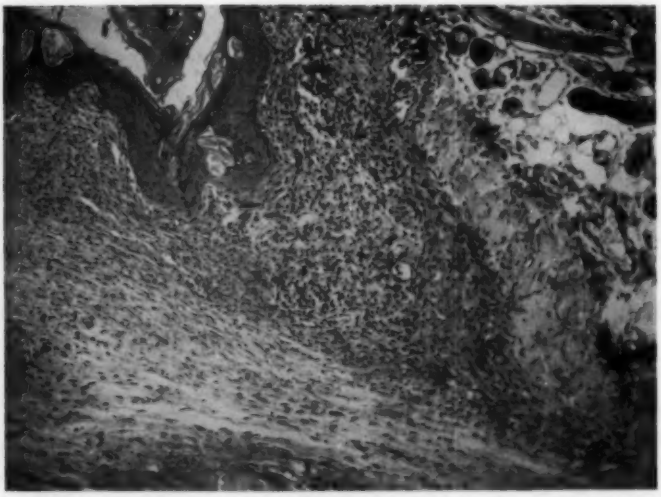

Fig. 1 (Experiment 1). —Control-group mouse. Wound area seven days after the incision. Complete organization of gap by regular granulation tissue with numerous capillaries. Reduced to 92% of mag. × 100.

Fig. 2 (Experiment 1). —Levan-group mouse treated by 2 × 10 mg. of native levan daily. Wound area seven days after the incision. Hemorrhage and gap not yet organized. Loose edematous tissue poor in capillaries and containing foam cells in lower part of figure. Reduced to 90% of mag. × 100.

the fibroblasts appeared to be disarranged, lying in different directions in a way similar to their appearance in tissue cultures (Fig. 5). The structure of the fibroblasts strengthened this analogy, as they appeared mostly thick, juicy, often with ramified processes. Although at three and four days most sections showed a normal amount of angioblastic proliferation, with formation of buds from existing capillaries, many of them acquiring lumina filled with erythrocytes, at six to nine days it was obvious that capillary proliferation was deficient. There were many fewer capillaries per unit of area around the wound, and the existing capillaries did not penetrate into the area of the incision. In many instances erythrocytes, originating probably from the incision, were found undamaged in the wound area. In many sections the capillary front lagged behind the fibroblastic advance, so that the loose, young connective tissue (containing fibroblasts and foamy histiocytes) contained very few or no capil-

Fig. 3 (Experiment 3).—Mouse treated by 2 × 15 mg. of native dextran daily. Six-day-old granulation tissue. Incomplete organization of hemorrhage. Foamy histiocytes. Paucity of capillary proliferation. × 150.

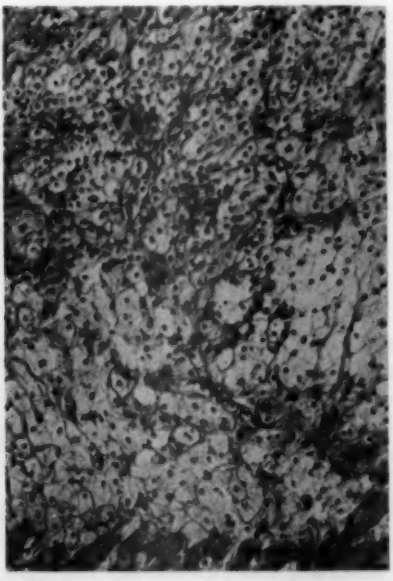

Fig. 4 (Experiment 3).—Mouse treated by 2 × 15 mg. of native dextran daily. Six-day-old granulation tissue. Foamy histiocyte reaction with few blood capillaries. × 145.

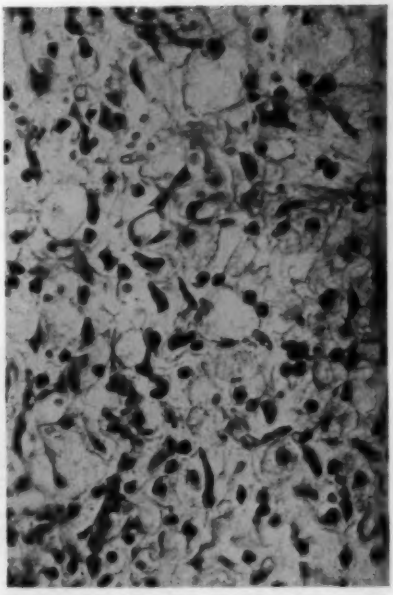

Fig. 5 (Experiment 3).—Mouse treated by 2 × 15 mg. of native dextran daily. Six-day-old granulation tissue. Edematous tissue with foamy histiocytes and disorganized arrangement of fibroblasts. × 400.

laries (Figs. 3, 4, and 5). The amount of stainable collagen and reticulum laid down in these mice was less than in the control animals.

As a rule, the thickness of the skin in the incised area and around it in the levanized mice was much less than in the controls. This phenomenon was particularly apparent in Experiment 2, in which the mice received 60 mg. of levan daily. The thinness of the dermis and its granulation tissue were discernible macroscopically, and this, taken together with the finding of the looseness of the reactive tissue in the animals treated by the polysaccharides, indicated how great the effect of these saccharides was on the proliferative phenomena.

There was no marked difference in the amount of inflammatory infiltration between the two groups. This finding is in contrast with the findings on the effect of levan in short-term experiments.[3]

No clear-cut effects of the high-polymer

saccharides on the regeneration of the epidermis could be detected.

Minor differences were noted between the animals treated by high-polymer levan and those treated by the comparable dextran: The inhibition of capillary proliferation seemed to be somewhat less marked in some of the dextran-treated animals, and the amount of collagen appeared slightly greater.

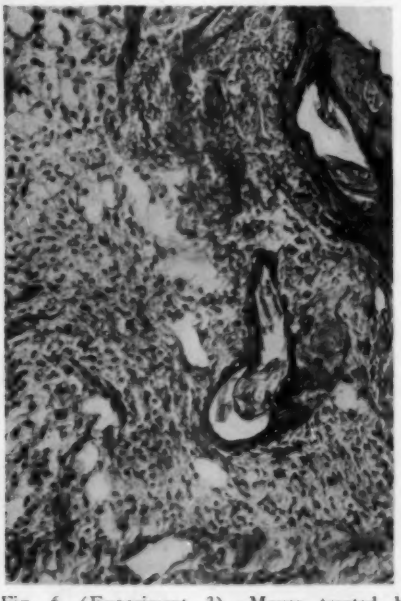

Fig. 6 (Experiment 3).—Mouse treated by 2 × 15 mg. of clinical leval daily. Six-day-old granulation tissue. Complete organization of wound by an edematous granulation tissue containing numerous foam cells. × 150.

In the mice treated with clinical levan foam cells were also abundant in the granulation tissue (Fig. 6). The fibroblasts appeared in most instances younger than in the controls; but their arrangement was regular and directed mainly toward the wounded area, and the amount of capillary proliferation and penetration was much more similar to the appearance in the non-treated animals than to that in the animals treated with the high polymers. The organization of the wound was apparently not retarded.

In the mice treated with clinical dextran

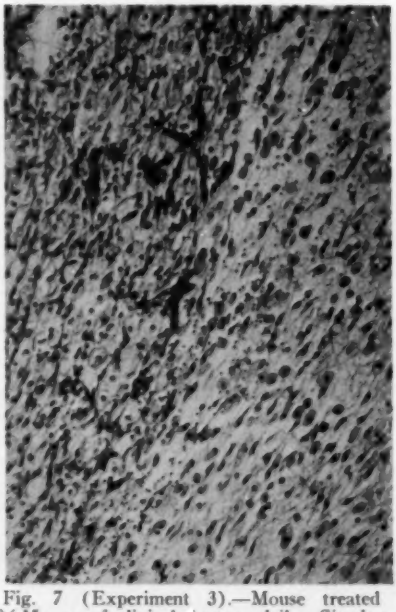

Fig. 7 (Experiment 3).—Mouse treated by 2 × 15 mg. of clinical dextran daily. Six-day-old granulation tissue. Complete organization of wound by an edematous, foam-cell-containing granulation tissue. Regular arrangement of fibroblasts. × 145.

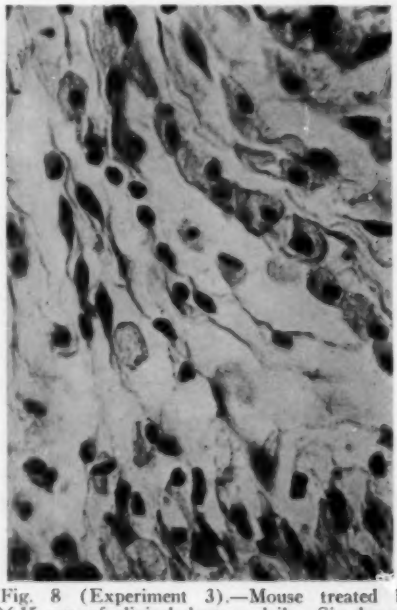

Fig. 8 (Experiment 3).—Mouse treated by 2 × 15 mg. of clinical dextran daily. Six-day-old granulation tissue. Histiocytes and capillary endothelial cells (?) undergoing foamy change. × 560.

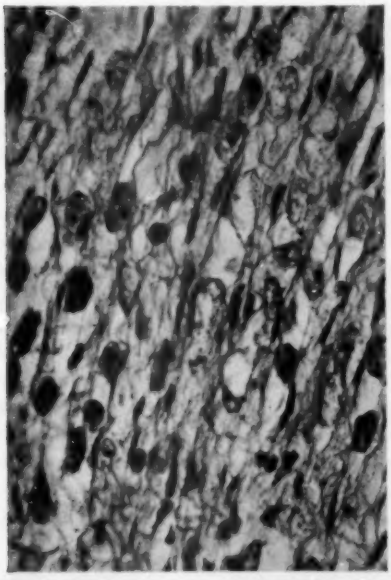

Fig. 9 (Experiment 3).—Mouse treated by 2 × 15 mg. of clinical dextran daily. Six-day-old granulation tissue. Foamy histiocytes. The larger ones are mostly PAS-negative; the cytoplasm of the smaller (bottom of the figure) stains intensely. PAS stain; × 370.

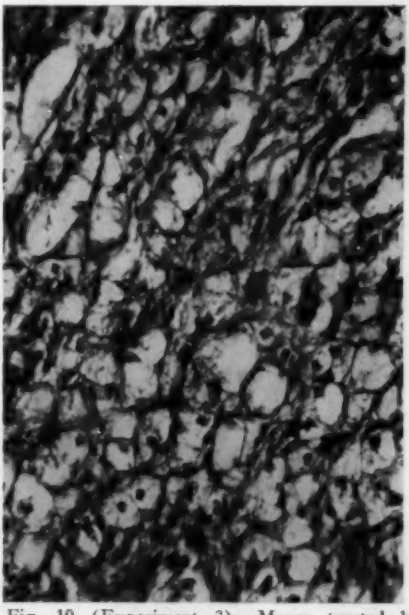

Fig. 10 (Experiment 3).—Mouse treated by 2 × 15 mg. of clinical dextran. Six-day-old granulation tissue. Between the foamy cells delicate strands of PAS-positive material. PAS stain; × 370.

the granulation tissue also contained numerous foam cells (Fig. 7). An example of foamy change occurring apparently in capillary endothelium can be seen in Figure 8. The findings were similar to those observed in the animals treated with clinical levan, but here the amount of capillary proliferation and the speed of organization were practically the same as those seen in the nontreated animals.

In sections stained by the PAS method various stages in the development of the foamy histiocytes could be discerned in animals treated by all the polysaccharides. Vacuoles in relatively small histiocytes stained with the PAS method. In larger histiocytes, containing many vacuoles as a rule, the content of these vacuoles was unstained (Fig. 9). In the animals treated with the low-polymer saccharides the ground substance contained delicate fibrils, which stained, as in the control animals, pink to red (Fig. 10). In the animals treated by the high polymers the ground substance

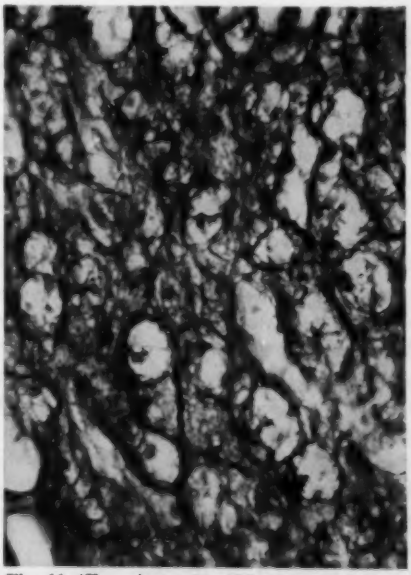

Fig. 11 (Experiment 3).—Mouse treated by 2 × 15 mg. of native levan daily. Six-day-old granulation tissue. Foamy histiocytes appear similar to cartilage cells with densely staining intercellular fibers enmeshing them. PAS stain; × 370.

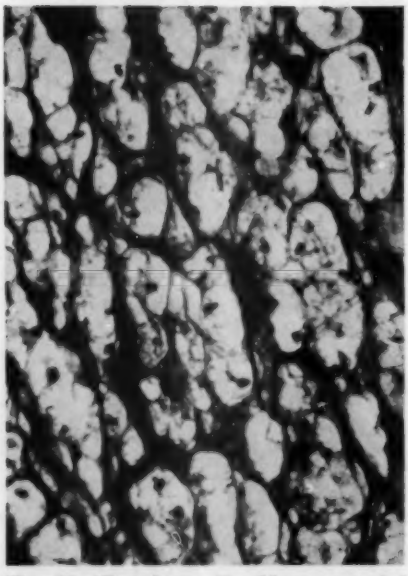

Fig. 12 (Experiment 3).—Mouse treated by 2 × 15 mg. of native dextran daily. Six-day-old granulation tissue. Similar to Figure 11, with an even closer likeness to cartilage. PAS stain.

stained heavily, appeared often fibrillar, and the clear, foamy cells were usually surrounded by a dense net of dark-purple material, which made them resemble PAS-stained cartilage cells (Figs. 11 and 12). The amount of reticulum between the foam cells was small in the animals treated by all the polysaccharides in comparison with that in the control group.

In sections stained by the colloidal iron method a difference could be detected between animals treated by the high polymers and those treated by the low polymers. There seemed to be more stainable material in the intercellular matter of the mice treated by the high polymers, and in the foamy histiocytes present in the granulation tissue. The appearance of the colloidal-iron-stainable material in the mice treated by the low polymers was similar to that of the controls.

Talcum-Injection Experiments. — On opening the abdomen of the nonlevanized mice injected with talcum, adhesions between the abdominal organs were invariably found. In many places organized talcum aggregates were noted within the mesentery or in the capsules of the abdominal organs. By scraping, the aggregates could be extracted from the tissues or exposed to the surface, but a jet of water delivered from a squeeze bottle could not disperse the aggregates.

In the levanized mice were many fewer adhesions between the organs (about half the animals did not have any). Talcum aggregates were found also in these animals within the mesentery and in the capsules of the abdominal organs. The surface of these aggregates was smooth and wet. On scraping the mesothelium, the talcum could be easily dispersed by a fine jet of water, which fact showed that the aggregates were covered by mesothelium, but were not properly organized.

Microscopically, the difference between the mice given native levan and the controls was essentially similar to that observed in the wound-healing experiments. In the nontreated animals, fibroblasts, capillaries,

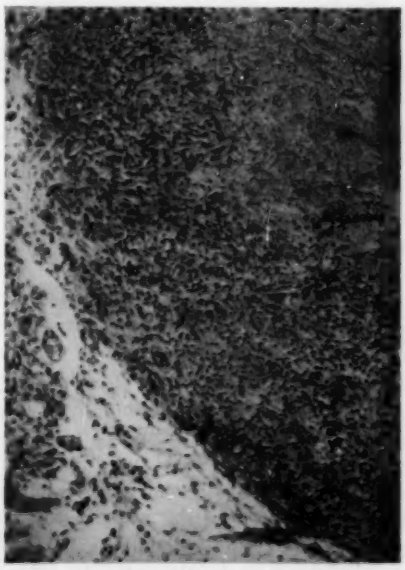

Fig. 13 (Experiment 4).—Control group mouse nine days after the intraperitoneal injection of a suspension of talcum. Partial organization of the talcum aggregate in the peritoneum. × 100.

and collagen fibers surrounded and penetrated deep into the talcum aggregates (Fig. 13). The granulomatous tissue contained a variable number of giant cells. In the levan-treated mice the talcum aggregates were also found within the tissue covered by mesothelium, but the granulomatous tissue capsule around them was very thin and the penetration of fibroblasts, capillaries, and collagen into the aggregates was minimal and involved only the periphery of the aggregates (Fig. 14). The granulation tissue appeared poorer in fibroblasts and giant cells and rich in intercellular substance. Many histiocytes appeared foamy, similar to those found in the wound-healing experiments.

Fig. 14 (Experiment 4).—Mouse given 30 mg. of levan daily. Peritoneal lesion nine days after the injection of the suspension of talcum. A foam-cell capsule around the talcum aggregate with scarce penetration. × 100.

It should be noted that in some experiments the animals were weighed at the beginning and the end of the experiment. No decrease in weight was noted during the treatment by polysaccharides.

Comment

High-polymer levan, and, to a slightly less extent, high-polymer dextran, when given in large doses, produced marked changes in the growth of granulation tissue. The growth of capillaries and of fibroblasts was inhibited, causing retardation in the absorption and organization of the exudate caused by the incision. The tissue appeared "edematous" and rich in intercullular ground substance, and contained many foamy histiocytes. The increase in intercellular material may have been due to a change in the constitution of the ground substance, probably caused by leakage of the polysaccharide into tissue spaces. The cartilage-like appearance of the foamy cells, which were surrounded by a strongly PAS-positive intercellular material, might be considered as an indication of an increased amount of neutral-polysaccharide material in the ground substance. It is interesting to note that the foamy appearance of the histiocytes was similar to that found in lipid-laden cells, although the content of the vacuoles could be stained in some cases by PAS or the colloidal-iron technique, and therefore probably represented polysaccharidic material.

The high polymers did not appear to inhibit the regenerative growth of the epidermis or the mesothelium.

The low-polymer homologues of the levan and dextran also caused changes in the structure of the granulation tissue. They did not, however, appreciably retard the organization of the exudate and the healing of the wound. The change was manifested mainly in the foamy appearance of the large histiocytes in and around the wound area.

High-polymer levan was probably somewhat more effective in the inhibition of the growth of granulation tissue than was the comparable dextran. The same may be said of the lower homologues. It is not clear whether the differences between the two carbohydrates are significant, and whether they are due to different metabolic pathways or to different physicochemical char-

acteristics of the compounds themselves.

Study of the PAS-stained sections indicates that the ground substance of the animals treated by the high polymers is very rich in polysaccharides. The morphologic resemblance of the "adult" foam cells to cartilage cells may be of biochemical significance, as in both cases the cells lie within a matrix rich in polysaccharides. It appears that these polysaccharides differ from the compounds injected, as they were not dissolved in the aqueous fixative. Whether the PAS-positive material in the ground substance represents the injected polysaccharide which was bound by tissue proteins or some other complex cannot at present be ascertained.

The appearance of the granulation-tissue fibroblasts in the animals treated by the high-polymer saccharides is reminiscent of the findings reported in avitaminosis C.[5] On the other hand, the combined picture of the effect of these polysaccharides on both acute [3] and chronic inflammatory phenomena shows a striking resemblance to the effects of adrenocortical hormones. It is well known that corticotropin and cortisone inhibit the phenomena of acute inflammation,† of wound healing,‡ and of the organization of silicotic aggregates, § whereas no clear-cut effect on the regeneration of epithelium could be constantly detected.[16]

This similarity may be more than coincidental if the effect of the adrenocortical hormones on the level of blood polysaccharides is taken into consideration. It is known that cortisone has a pronounced effect on the metabolism of the muco-polysaccharides of the ground substance and of the blood.‖ Administration of corticotropin or cortisone to patients with rheumatic fever and rheumatoid arthritis caused a decrease in the amount of mucoproteins in the blood.¶ Cortisone was shown to inhibit sulfate fixation in the ground substance,[21] which fixation goes on actively in granulation tissue.# It has also been suggested that there exists an antagonism between the acid and the neutral polysaccharides of the ground substance.* It may, therefore, be surmised that the administration of high-polymer polysaccharides elicits changes in the ground substance which are comparable to those produced by the administration of cortisone. The effects of cortisone administration would depend, in such a case, on physiological changes which are neatly copied by the administration of high-polymer polysaccharides; i.e., the cortisone would cause an increase in the amount of neutral polysaccharides present in the tissue spaces, possibly by inhibiting sulfate fixation. The administration of the high-polymer polysaccharides would elicit a similar change by flooding the tissue spaces with neutral polysaccharides. It is also interesting to speculate to what extent the administration of high-polymer polysaccharides reproduces the state of affairs existing in some states of disease, such as diabetes complicated by changes in the blood vessels, where there is a known tendency of infections to spread, and retardation of wound healing and in which there is a high level of serum mucopolysaccharides.[26]

The basic problem as to whether the phenomena described above were caused by a supposed increase in the absolute amount of neutral polysaccharides in the ground substance, or whether a physiological balance between polar and nonpolar polysaccharide has been disturbed, cannot be answered as yet.

Summary

High-polymer (native) dextran and levan given in high doses, preferably every 12 hours, inhibit the formation of granulation tissue in wounds of the skin and around talc particles in the peritoneal cavity. The inhibition is represented histo-

† References 6-7.
‡ References 8-12.
§ References 13-15.
‖ References 17-18.
¶ References 19-20.
# References 22-23.
* References 24-25.

logically by a decrease in the proliferation and penetration of capillaries, inhibition of formation and disarrangement of fibroblasts, increased amount of intercellular substance, and a foamy transformation of histiocytes.

The administration of comparable doses of the partially degraded polysaccharides (of a molecular weight which corresponds to that of dextran in use as a plasma-volume expander) does not affect appreciably the healing of wounds. These polysaccharides, like the native substances, caused a foamy change in the histiocytes of the granulation tissue.

The similarity between the effects of the high polymers and of cortisone on the phenomena of acute inflammation and repair is stressed. It is suggested that the adrenocortical hormones may exert their effects on these phenomena through their action on the naturally occurring polysaccharides of the body.

Miss F. Aslan and Mrs. J. Tarabulus rendered technical assistance, and Mrs. H. Weinman did the microphotography.

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News and Comment

PERSONAL NEWS

Dr. John C. Bugher given Harold Taylor Ricketts Award.—Dr. John C. Bugher, director of medical education and public health, Rockefeller Foundation, New York, formerly of the Department of Pathology, University of Michigan, was given the Howard Taylor Ricketts Award for 1956 at the University of Chicago on May 9. He discussed the problem of Changing Patterns in the Public Health.

PERSONAL NEWS

Address by Shields Warren.—Dr. Shields Warren, of Boston, addressed the First Scientific Assembly of the Florida Medical Association in Miami Beach under the title "The First Decade of Atomic Medicine."

PERSONAL NEWS

Appointment of Dr. Stuart W. Lippincott.—Stuart W. Lippincott, professor of pathology at the University of Washington School of Medicine, has accepted a senior appointment in the division of experimental pathology at Brookhaven National Laboratory, Upton, N. Y., and is pathologist for the Brookhaven hospital.

Dr. Richard W. Tiecke Elected President of American Academy of Oral Pathology.—Dr. Richard W. Tiecke, associate professor of pathology at the Northwestern University Dental School, was elected president of the American Academy of Oral Pathology at its recent annual meeting in Washington, D. C.

Books

The Shoulder and Environs. By James E. Bateman, M.D., F.R.C.S.(C). Price, $16.25. pp. 565, with 376 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1955.

Dr. Bateman's text is an outstanding contribution to orthopedic literature. He has covered the affections of and about the shoulder in a logical and systematic fashion, beginning with the functional anatomy and physiology and continuing through a thorough consideration of the various pathologic processes which may involve the region. Charts and outlines of the main points in differential diagnosis and therapy are contained in the appropriate chapters. Careful attention is given to the cervical, thoracic, cardiac, and abdominal problems which may manifest themselves in shoulder-arm symptoms. Especially noteworthy for students are the sections on roentgenographic and physical examination. These rather difficult subjects are presented with clarity and ample illustration. Of general interest are the brief descriptions of the more important surgical procedures performed about the shoulder and the final section on management and assessment of disability.

"The Shoulder and Environs" is a book containing something of value for the student, practitioner, and specialist alike.

Antimetabolites and Cancer. By C. P. Rhoads, M.D., Editor. Price, $5.75. Pp. 318, with 50 illustrations. American Association for the Advancement of Science, 1515 Massachusetts Ave., N .W., Washington 5, D.C., 1955.

The symposium, Antimetabolites and Cancer, outlines some of the recent advances in the chemotherapy of cancer, correlating information from several areas bearing on this problem. The editor's list includes many persons outstanding in their subject fields. Among them are D. W. Wooley, H. E. Skipper, J. H. Burchenal, G. B. Brown, S. Weinhaus, G. H. Hitchings, and W. Shive, to mention just a few. The first paper presents recent information concerning the metabolic pathways of energy metabolism of tumors. Following it are numerous reports dealing with antimetabolic activity, including such topics as the inhibitory effect of normal metabolites, as well as of purine, pyrimidine, and vitamin analogues. The problem and mechanism of the development of drug resistance and its importance in therapy are ably discussed. In the highly interesting discussion by C. P. Rhoads, he reminds us that in the field of cancer chemotherapy there is at least one rational basis for the preparation of therapeutic agents. This lies in the fact that cancer cells have a variation in their reproductive organization, requiring accordingly that the chromosomes and their DNA components must be different. That the DNA constitution of different cells is variable has been demonstrated by the differential rates of incorporation of isotopically labeled purines by different types of cells, and confirmed by studies of the uptake of C^{14} formate.

The fact that anti-folic- and anti-nucleic-acid precursors selectively inhibit neoplastic cells indicates that the differences in DNA can be made use of in the destruction of the abnormal cells and not the others.

Each paper is valuable as a unit, and the combination of many different types of studies enables the reader to direct his thought along channels that will converge on a better understanding of antimetabolites and the treatment of cancer.

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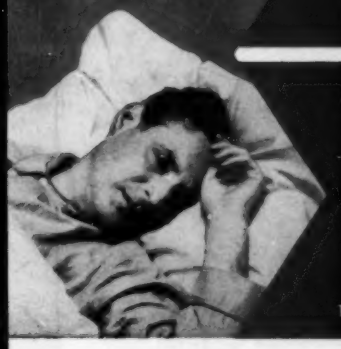

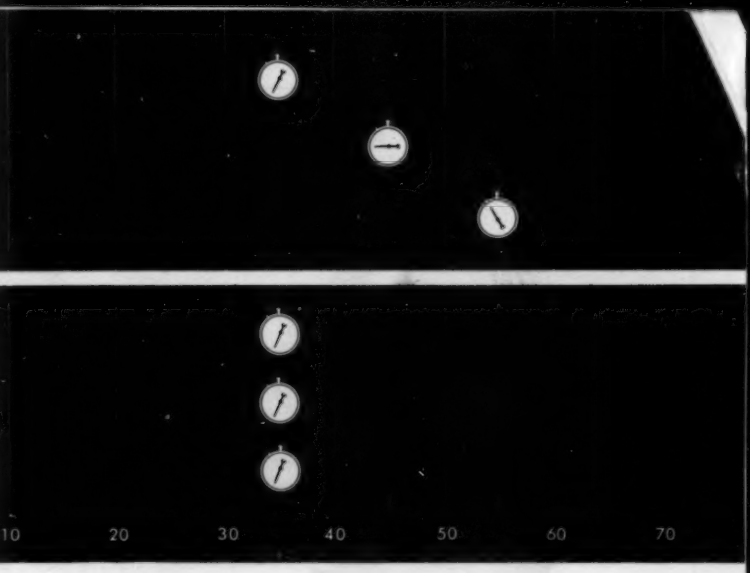

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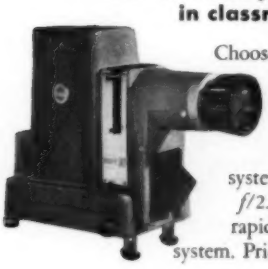

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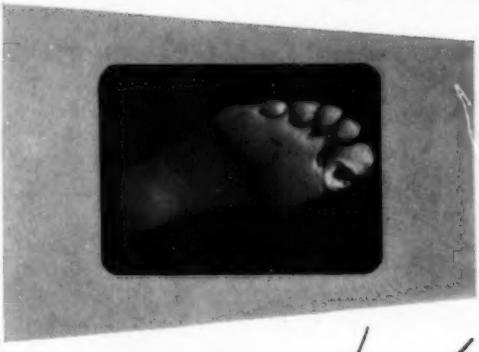

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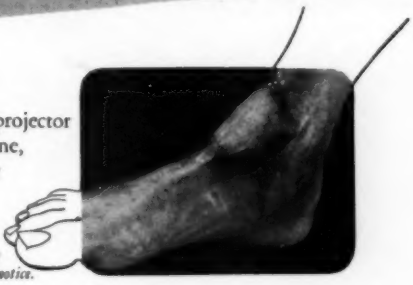

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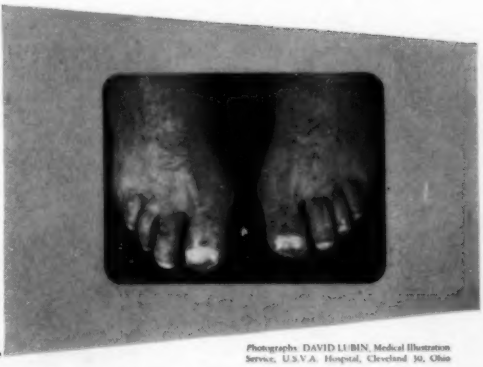

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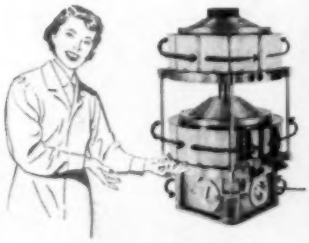

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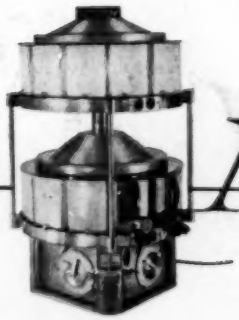

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